(54) Title: RELAXIN-CHIMERIC POLYPEPTIDES AND THEIR PREPARATION AND USE

	B-chain	A-chain
Relaxin-3	RAAPYGVRLCGREFIRAVIFTCGGSRW	DVLAGLSSSCCKWGCSKSEISSLC
R3/R1	RAAPYGVRLCGREFIRAVIFTCGGSRW	RPYVALFEKCCLIGCTKRSLAKYC
R3/R2	RAAPYGVRLCGREFIRAVIFTCGGSRW	QLYSALANKCCHVGCTKRSLARFC
R3/I3	RAAPYGVRLCGREFIRAVIFTCGGSRW	ATNPARYCCLSGCTQQDLLTLC
R3/I4	RAAPYGVRLCGREFIRAVIFTCGGSRW	FDPFCCEVICDDGTSVKLC
R3/I5	RAAPYGVRLCGREFIRAVIFTCGGSRW	DLQTLCCTDGCSMTDLSALC
R3/I6	RAAPYGVRLCGREFIRAVIFTCGGSRW	GYSEKCCLTGCTKEELSIAC
R3/I	RAAPYGVRLCGREFIRAVIFTCGGSRW	GIVEQCCTSICSLYQLENYC
R3/A	RAAPYGVRLCGREFIRAVIFTCGGSRW	GLYENCCLSICDDYEIEKAC

(57) Abstract: Chimeric polypeptides of relaxin-3, prepropolypeptides thereof, polynucleotides encoding such polypeptides, and associated expression vectors and host cells are described. The polypeptides may be used to prepare receptor-ligand complexes with GPCR135 or GPCR142, which may be used in assay methods.

RELAXIN-3 CHIMERIC POLYPEPTIDES AND THEIR PREPARATION AND USE

FIELD OF THE INVENTION

The invention generally relates to biologically active relaxin-3 chimeric polypeptides and their preparation and use, e.g., as assay reagents.

BACKGROUND OF THE INVENTION

Relaxin-3 (also known as INSL7) has been found to be a ligand for the G-protein coupled receptors (GPCRs) GPCR135 and GPCR142. See US Provisional Application No. 60/493,941, filed 7 August 2003, and International Application No. PCT/US2004/005666, filed 25 February 2004, the disclosures of which are incorporated by reference herein.

GPCRs are transmembrane receptor proteins that are responsible for the transduction of a diverse array of extracellular signals, including hormones, neurotransmitters, peptides, lipids, ions, light, odorants, nucleotides, fatty acid derivatives, and other chemical mediators. See, e.g., WIPO Publication No. WO 02/00719. GPCRs are of particular importance to drug discovery because they have been established as excellent drug targets: they are the targets of 50% of marketed drugs. An increasing number of diseases have been found to be associated with GPCRs. Drugs targeting GPCRs have been used to treat a wide range of disorders from cardiovascular to gastro-intestinal to CNS and others (Wilson et al., 1998, *British J. of Pharmacology*, 125:1387-1392).

The GPCR-mediated signal transduction event is often initiated upon binding of a specific ligand to the GPCR. Each GPCR is composed of an extracellular N-terminal domain, seven distinct transmembrane segments, and an intracellular C-terminal domain. Binding of the ligand

to an extracellular N-terminal domain, transmembrane domain, or intracellular loop of a GPCR results in a conformational change that leads to activation of intracellular heterotrimeric GTP-binding proteins (G proteins) associated with the GPCR. These activated G proteins in turn mediate a variety of intracellular responses that regulate cell physiology. Therefore, the ligand provides means of elucidating the physiological function of the GPCR as well as methods of screening for compounds that regulate the signal transduction activity of the GPCR.

At present, only about 200 GPCRs are classified as known GPCRs that are activated by around 70 known ligands. Through sequence analyses, it was discovered that GPCRs belong to one of the largest superfamilies of the human genome: evaluated at over 1000 genes encoding GPCRs (Civelli et al., 2001, *Trends in Neurosciences*, 24:230-237). A large number of putative GPCRs are described as orphan receptors because their natural ligands are unknown. Some of these uncharacterized orphan GPCRs may be useful as therapeutic targets. The identification of the specific ligand to a GPCR is the key to harnessing the potential therapeutic benefits of these orphan GPCRs (Howard et al., 2001, *Trends in Pharmacological Sciences*, 22:132-140).

As noted above, relaxin-3 (also known as INSL7) has been found to be a ligand for GPCR135 as well as for GPCR142. See Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764; and Liu et al., 2003, *Journal of Biological Chemistry*, 278:50765-50770. Relaxin-3 is a member of the insulin/relaxin superfamily. Members in this family are characterized by peptide subunits (A-chain and B-chain) linked by three disulfide bonds. Two of the three disulfide bonds are inter-subunit bonds and

another one is an intra-chain bond in the B-chain. In the family, insulin, IGF1, and IGF2 have been reported to be involved in the regulation of glucose metabolism and signal through tyrosine kinase/growth factor receptors, which are single transmembrane receptors. Another member of the relaxin/insulin superfamily is Insulin-Like (INSL) 5 (Conklin et al. 1999, *Genomics*, 60(1):50-56), which is believed to be a selective ligand for GPCR142 (see, e.g., U.S. Provisional Application No. 60/580,083, the disclosure of which is incorporated by reference herein). Two other members in the family are relaxin and INSL3, ligands for LGR7 and/or LGR8, which are GPCRs with leucine-rich repeats at the N-terminal extra-cellular domain. Relaxin-3 was also reported to be an additional ligand for LGR7.

The GPCR142 expression pattern is distinct from that of GPCR135 and relaxin-3 with an abundant peripheral tissue distribution in addition to expression in the brain (Liu et al., 2003, *Journal of Biological Chemistry*, 278(50):50765-50770)). We have found that GPCR142 is highly conserved among human, monkey, cow, and pig, but is less conserved in the mouse, and a pseudo-gene exists in the rat despite a fair degree of conservation of relaxin-3 genes in both rodent species, suggesting that GPCR142 has a distinct function from GPCR135 and diminished function in the rodents. All the physiological functions of GPCR135 and GPCR142 remain to be fully elucidated. *In vivo* administration of relaxin-3 also activates LGR7, which is expressed in both the brain and periphery (Hsu et al., 2000, *Mol. Endocrinol.*, 14:1257-1271; Hsu et al., 2002, *Science*, 295:671-674; Tan et al., 1999, *Br. J. Pharmacol.*, 127:91-98). Thus, *in vivo* study of GPCR135 or GPCR142 functions has been confounded by

the lack of selective pharmacological tools for these two receptor subtypes.

SUMMARY OF THE INVENTION

Biologically active chimeric polypeptides of relaxin-3 have now been discovered.

Thus, in one general aspect, the invention relates to a biologically active relaxin-3 chimeric polypeptide comprising a relaxin-3 B-chain and a relaxin/insulin polypeptide family member A-chain. In preferred embodiments, the A-chain is a relaxin-1 A-chain, a relaxin-2 A-chain, an insulin-like 3 A-chain, an insulin-like 4 A-chain, an insulin-like 5 A-chain, or an insulin-like 6 A-chain. More preferably, the A-chain is an insulin-like 5 A-chain. Even more preferably, the chimeric peptide is human relaxin-3 A-chain/human INSL5 B-chain having an amino acid sequence as set forth in SEQ ID NO:23.

The invention is also directed to polynucleotides encoding the above polypeptides. Thus, in one general aspect the invention relates to a relaxin-3 chimeric pre-propolypeptide comprising a relaxin B-chain joined by a first peptide linkage to a relaxin C-chain, the C-chain further joined by a second peptide linkage to a relaxin/insulin family member A-chain, and a protease cleavage site inserted at at least one location selected from the second peptide linkage between the A-chain and C-chain and the first peptide linkage between the C-chain and the B-chain of the relaxin-3 chimeric pre-propolypeptide. In a preferred embodiment, the protease cleavage site, preferably a furin cleavage site, is inserted at the peptide linkage joining the C-chain and A-chain. Preferably, the polynucleotide has a nucleotide sequence selected from the polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:7 and

complements thereof which hybridize under stringent conditions to such polynucleotide.

In another aspect, the invention is directed to pre-propolypeptides encoded by such polynucleotides.

In yet another aspect, the invention relates to expression vectors suitable for expression in recombinant host cells, the vector comprising the above-mentioned polynucleotides.

Another aspect of the invention relates to host cells comprising the expression vectors.

An additional general aspect of the invention relates to a receptor-ligand complex comprising a receptor component comprising GPCR135 or GPCR142, the receptor component bound to a ligand component comprising a biologically active relaxin-3 chimeric polypeptide. In preferred embodiments, the polypeptide corresponds to SEQ ID NO:23 and bears a radioisotope label. In other preferred embodiments, the receptor component comprises GPCR135 and/or GPCR142 associated with isolated cell membranes or lipid vesicles. Preferably, the receptor and ligand components are each in a substantially pure form.

In a further general aspect, the invention relates to a process of producing a mature relaxin-3 chimeric polypeptide from a recombinant cell, comprising: (a) transforming or transfecting a host cell with a first expression vector as described above and a second vector expressing a protease for cleaving a relaxin-3 chimeric pre-propolypeptide at an inserted protease cleavage site; and growing the host cell so that both the relaxin-3 chimeric pre-propolypeptide and the protease are expressed, whereby the protease cleaves a peptide linkage at the inserted protease cleavage site on the relaxin-3 chimeric pre-propolypeptide, yielding a mature relaxin-3

chimeric polypeptide. Preferably the process further comprises: expressing a receptor component on the cell surface of a host cell that has been transformed or transfected with an expression vector encoding GPCR135 or GPCR142; and complexing the receptor component with the relaxin-3 chimeric polypeptide.

The invention also generally relates to a method of identifying a compound that modulates a biological activity of GPCR135 and/or GPCR142, comprising: (a) contacting a test sample comprising a compound with an assay reagent comprising the receptor and a biologically active relaxin-3 chimeric polypeptide; (b) then determining the biological activity of the receptor; and (c) comparing the biological activity determined with a control measurement obtained by contacting a control sample not containing the compound with the assay reagent. Preferably, the receptor is a component of a biological sample derived from a rat or a human. Determination of the biological activity may comprise identifying the formation of a receptor-ligand complex (e.g., using autoradiography) or measuring a second messenger response (e.g., by intracellular calcium ion concentration or intracellular cAMP concentration). For example, the receptor may be associated with isolated cell membranes from a GPCR135 host cell or a GPCR142 host cell, and the biological activity may be determined by measuring the amount of protein phosphorylation of the isolated membranes using a γ-phosphate labeled GTP molecule such as ^{35}S-GTPγS, ^{33}P-GTPγP, or ^{32}P-GTPγP.

In another aspect, the invention provides a method of identifying a compound that binds to at least one receptor selected from GPCR135 and GPCR142, comprising: (a) contacting the receptor with a test compound and with a labeled relaxin-3 chimeric polypeptide; (b) determining

the amount of the labeled relaxin-3 chimeric polypeptide that binds to the receptor; and (c) comparing the amount determined in step (b) with a control measurement obtained by contacting the receptor with the labeled relaxin-3 chimeric polypeptide in the absence of the test compound.

Other aspects, features and advantages of the invention will be apparent from the following disclosure, including the detailed description of the invention and its preferred embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show the activation of GPCR135 and GPCR142 by relaxin-3 B-chain, but not of LGR7 or LGR8. In Fig. 1A, SK-N-MC/β-gal cells expressing human GPCR135 were stimulated with relaxin-3, relaxin-3 B-chain, porcine relaxin, or INSL3 at various concentrations to inhibit the forkolin stimulated β-galactosidase expression. Forskolin was added to cells at a final concentration of 5 μM to stimulate the β-galactosidase expression. Fig. 1B depicts results for SK-N-MC/β-gal cells expressing human GPCR142 stimulated as in Fig. 1A. In Fig. 1C, SK-N-MC/β-gal cells expressing human LGR7 were stimulated with relaxin-3, relaxin-3 B-chain, porcine relaxin, and INSL3 at various concentrations to induce the β-galactosidase expression. The intracellular β-galactosidase activities were measured by a colorimetric assay using CRGP as the substrate and reading the absorbance at a wavelength of 570 nm. For Fig. 1D, SK-N-MC/β-gal cells expressing human LGR8 were stimulated as in Fig. 1C.

Figure 2 shows the amino acid sequences of human relaxin-3 and relaxin-3 chimeric polypeptides. A chimeric polypeptide is a hybrid molecule containing

specific units from different proteins or polypeptides.
All chimeric polypeptides depicted have the identical B-
chain from human relaxin-3. The chimeric polypeptides
R_3/R_1, R_3/R_2, R_3/I_3, R_3/I_4, R_3/I_5, R_3/I_6, and R_3/I possess A-
chain sequences from human relaxin-1, relaxin-2, INSL3,
INSL4, INSL5, INSL6, and insulin, respectively. R3/A
possesses an A-chain in which the conserved cysteine
residues are retained and the remainder of the amino acid
sequence is arbitrarily assigned.

Figures 3A-3D illustrate the characterization of
various relaxin-3 chimeric polypeptides using radioligand
binding assays. Different relaxin-3 chimeric
polypeptides were characterized for their receptor
binding properties for human recombinant GPCR135 (Fig.
3A), GPCR142 (Fig. 3B), LGR7 (Fig. 3C), or LGR8 (Fig.
3D). For GPCR135, GPCR142, and LGR7, $[^{125}I]$-relaxin-3 was
used as the radioligand at a final concentration of 100
pM. Different unlabeled peptides were used in the binding
assay as the competitors. For LGR8, $[^{125}I]$-INSL3 was used
as the radioligand at a final concentration of 100 pM.
Different unlabeled peptides including relaxin-3 chimeras
at various concentrations were added to the binding assay
as the competitors. The IC_{50} values of different chimeras
to different receptors are provided in Table 1 below.
Data represent mean ± SEM (n = 3).

Figures 4A-4D illustrate the evaluation of the
agonist activity of relaxin-3 chimeric polypeptides in
biofunctional assays. Different chimeric polypeptides at
various concentrations were added to SK-N-MC/β-gal cells
expressing human GPCR135 (Fig. 4A) or GPCR142 (Fig. 4B)
to inhibit forskolin stimulated β-galactosidase
expression. Human relaxin-3 was used as the positive
control. In parallel, chimeric polypeptides at various
concentrations were added to SK-N-MC/β-gal cells

expressing human LGR7 (Fig. 4C) and LGR8 (Fig. 4D) to stimulate β-galactosidase expression. Relaxin-3 and INSL3 were used as positive controls for LGR7 and LGR8, respectively. Intracellular β-galactosidase activity was measured by a colorimetric assay using CRGP as the substrate and reading the absorbance at wavelength of 570 nm. The EC_{50} values of different chimeras to the four receptors are listed in Table 2. Data represent mean ± SEM (n = 3).

Figures 5A-5D illustrate a comparative analysis of the binding specificities of [^{125}I]-relaxin-3 and [^{125}I]-R_3/I_5 for GPCR135 and LGR7. COS-7 cells in 6-well culture dishes were transfected with human GPCR135 and LGR7, respectively. Mock transfected COS-7 cells were used as the negative controls. [^{125}I]-relaxin-3 (Fig. 5A) or [^{125}I]-R_3/I_5 (Fig. 5B) was added to cells at a final concentration of 100 pM either in the absence or presence of 1 uM of unlabeled relaxin-3 to determine total binding and non-specific binding, respectively. Fig. 5C depicts an autoradiogram of [^{125}]-relaxin-3 binding in rat brain sagittal section. Fig. 5D depicts an autoradiogram of non-specific binding of [^{125}]-relaxin-3 as determined in the presence of 100 nM of unlabeled relaxin-3. Fig. 5E depicts an autoradiogram of [^{125}]-R_3/I_5 binding in rat brain sagittal section. Fig. 5E depicts non-specific binding of [^{125}]-R_3/I_5 as determined in the presence of 100 nM of unlabeled relaxin-3. CX = Cortex, OB = Olfactory Bulb, SC = Superior Colliculus.

Figure 6 provides an amino acid sequence comparison of members of the insulin/relaxin family. The two conserved positively charged amino acids in the B-chains of relaxin-1 (SEQ ID NO:80), relaxin-2 (SEQ ID NO:81), relaxin-3 (SEQ ID NO:28), INSL3 (SEQ ID NO:82), INSL4 (SEQ ID NO:83), INSL5(SEQ ID NO:84), and INSL6 (SEQ ID

NO:85) are highlighted in bold letters. The asterisk (*)
denotes that B-chain and A-chain termini for INSL4,
INSL5, and INSL6 are based on predictions. The amino
acid sequences of insulin (SEQ ID NO:86), insulin-like
growth factor (IGF) I (SEQ ID NO:87), and IGF2 (SEQ ID
NO:88) are also provided.

Figures 7A-7B illustrate a characterization of the
selectivity of the R3/I5 chimera as a ligand for rat
GPCR135 receptors compared to rat LGR7 in recombinant
cells. The R3/I5 chimera was characterized for its
receptor binding properties for rat recombinant GPCR135
(Fig. 7A) or LGR7 (Fig. 7B) receptors. [^{125}I]-R3 was used
as tracer (100 pM). Data shown are the mean values $\pm$ SEM
of triplicate experiments.

Figures 8A-8C depict a pharmacological
characterization of [^{125}I]-R3/I5 binding sites in
autoradiograms from a series of coronal sections at the
level of the rat superior colliculus (left hemisphere):
7 pM [^{125}I]-R3/I5 (Fig. 8A) was competed with 10 nM R3
(Fig. 8B) or 100 nM Relaxin (Fig. 8C). In the figures,
SC = superior colliculus; I.P. = interpeduncular nucleus;
AHi = amygdalohippocampal area.

Figure 9 shows displacement of [^{125}I]-R3/I5 binding
sites to the rat superior colliculus by unlabeled R3 as
determined by quantitative autoradiography. Consecutive
coronal sections were incubated with 7 pM of [^{125}I]-R3/I5
in the presence of increasing concentrations of R3. Data
are the means $\pm$ SEM of triplicate experiments.

Figures 10A-10F'' illustrate a rostro-caudal
distribution of GRPC135 mRNA (Figs. 10A, 10B, 10C, 10D,
10E, and 10F) and [^{125}I]-R3/I5 binding sites (Figs. 10A',
10B', 10C', 10D', 10E', and 10F') in autoradiograms from
a series of coronal sections through rat front and mid
brain (left hemisphere). Non-specific binding was

determined in the presence of 100 nM R3 (Figs. 10A'',
10B'', 10C'', 10D'', 10E'', and 10F''). In the figures,
Amyg = amygdala; BNST = bed nucleus of stria terminalis;
CA3 = field of CA3 of hippocampus; CM = central thalamic
nucleus; DG = dentate gyrus; Ha = habenula; LH = lateral
hypothalamus; MCx = motor cortex; OB = olfactory bulb; ON
= olfactory nucleus; PVA = paraventricular thalamic
nucleus; PVN = paraventricular hypothalamic nucleus; S =
septum; SCx = somatosensory cortex; SO = supraoptic
nucleus.

Figures 11A-11F'' illustrate a rostro-caudal
distribution of GRPC135 mRNA (Figs. 11A, 11B, 11C, 11D,
11E, 11F) and [^{125}I]-R3/I5 binding sites (Figs. 11A',
11B', 11C', 11D', 11E', 11F') in autoradiograms from a
series of coronal section through the rat hind brain
(left hemisphere). Non-specific binding was determined
in the presence of 100 nM R3 (11A'', 11B'', 11C'', 11D'',
11E'', 11F''). AHi = amygdalohippocampal area; DG =
dentate gyrus; DRD = dorsal raphe dorsal part; IC =
inferior colliculus; IP = interpeduncular nucleus; NI =
nucleus incertus; PAG = periaqueductal gray area; SC =
superior colliculus; Sol = nucleus of solitary tract; Sp5
= Spinal trigeminal tract; TCx = temporal cortex; VCx =
visual cortex.

DETAILED DESCRIPTION OF THE INVENTION AND ITS PREFERRED
EMBODIMENTS

    The disclosures of all publications cited herein, or
corresponding to accession numbers cited herein, are
hereby incorporated by reference. Unless defined herein
or otherwise indicated below, all technical and
scientific terms used herein have the same meaning as
commonly understood in the art.

    As used herein, the terms "comprising", "including",
and "containing" are used in their open, non-limiting
sense.

    The following are abbreviations that may at times be
used in this specification:

    bp = base pair

    Ca^{2+} = calcium ion

    cAMP = cyclic adenosine monophosphate

    cDNA = complementary DNA

    CNS = central nervous system

    kb = kilobase; 1000 base pairs

    kDa = kilodalton; 1000 dalton

    GPCR = G protein coupled receptor

    G protein = GTP - binding protein

    GTP = guanosine 5'-triphosphate

    nt = nucleotide

    PAGE = polyacrylamide gel electrophoresis

    PCR = polymerase chain reaction

    SDS = sodium dodecyl sulfate

    SiRNA = small interfering RNA

    UTR = untranslated region

    As noted above, certain general embodiments of the
invention relate to isolated biologically active relaxin-
3 chimeric polypeptides, polynucleotides that encode
immature pre-propolypeptides from which the relaxin-3
chimeric polypeptides are derived, expression vectors

comprising such polynucleotides, and recombinant host cells transfected or transformed by such vectors.

Exemplary relaxin-3 polypeptides include relaxin-3 orthologs that have been identified in human (GenBank protein Accession No.: NP_543140), rat (SEQ ID NO:51) (Burazin et al., 2002, *J. Neurochem.*, 82: 1553-1557; GenBank protein Accession No. NP_733767; 76.4% sequence identity to that of human), mouse (SEQ ID NO: 49)(Bathgate et al., 2002, *J. Biol. Chem.*, 277: 1148-1157; GenBank protein accession number XP_146603; 78.7% sequence identity to that of human), and other animals, including pig and monkey. These polypeptides can be used as sources of relaxin-3 units to form suitable chimera.

A "relaxin-3 chimeric polypeptide", or "ligand component" means a mature relaxin-3 chimeric polypeptide, or active fragment thereof, which is a secreted protein containing a relaxin-3 polypeptide B-chain and a relaxin/insulin family member A-chain, linked by disulfide bridges or bonds, or a functionally equivalent fragment of such mature polypeptide or secreted protein.

A "relaxin/insulin family member" is, in reference to a specified polypeptide, a polypeptide that bears functional homology (e.g., has similar biological activity, such as association with similar types of receptors) or amino acid sequence homology. Exemplary relaxin/insulin family member polypeptides include relaxin polypeptides, such as relaxin-1, relaxin-2, relaxin-3, and insulin-like polypeptides, also termed INSLs, such as insulin-like 3 (INSL3), insulin-like 4 (INSL4), insulin-like 5 (INSL5), and insulin-like 6 (INSL6). Exemplary orthologs of relaxin/insulin family members include orthologs identified in the accompanying Sequence Listing. Thus, in exemplary embodiments, the relaxin-3 chimeric polypeptides are derived from a

relaxin-3 component and a relaxin/insulin family member
component from any of the relaxin/insulin family member
orthologs described above.

A "relaxin-3 chimeric pre-propolypeptide" means a
precursor of a relaxin-3 chimeric polypeptide.

Preferred embodiments of a relaxin-3 chimeric pre-
propolypeptides and polypeptides include, e.g., (1) pre-
propolypeptides and mature polypeptides that have at
least 50% more preferably at least 60%, more preferably
at least 70%, more preferably at least 80%, more
preferably at least 90%, and even more preferably 100%
amino acid sequence identity to the following chimera (B-
chain/A-chain): human relaxin-3/relaxin-1 pre-
propolypeptide (SEQ ID NO:11) and mature polypeptide (SEQ
ID NO:19), a human relaxin-3/relaxin-2 pre-propolypeptide
(SEQ ID NO:12) and mature polypeptide (SEQ ID NO:20), a
human relaxin-3/INSL3 pre-propolypeptide (SEQ ID NO:13)
and mature polypeptide (SEQ ID NO:21), a human relaxin-
3/INSL4 pre-propolypeptide (SEQ ID NO:14) and mature
polypeptide (SEQ ID NO:22), a human relaxin-3/INSL5 pre-
propolypeptide (SEQ ID NO:15) and mature polypeptide (SEQ
ID NO:23), or a human relaxin-3/INSL6 pre-propolypeptide
(SEQ ID NO:16) and mature polypeptide (SEQ ID NO:24); or
(2) pre-propolypeptides or polypeptides that are capable
of binding to antibodies, e.g., polyclonal or monoclonal
antibodies, raised against such relaxin-3 chimeric
polypeptides.

A polypeptide or polynucleotide that is
"biologically active" exerts a biological activity as
determined *in vivo* or *in vitro*. Such activities can be a
direct activity, such as an association with or an
enzymatic activity on a second protein, or an indirect
activity, such as a cellular signaling activity mediated
by interaction of the protein with a second protein.

Biological activities may be measured according to standard techniques, such as the exemplary techniques described below.

For example, a biological activity possessed by a preferred relaxin-3 chimeric polypeptide described below is its ability to bind with high affinity to a GPCR135 or a GPCR142, while not binding to an LGR7 or LGR8, thereby selectively stimulating either GPCR135 or GPRC142 and signal transduction events governed therewith. An exemplary biological activity of GPCR135 or GPCR142 is that, upon binding to a relaxin-3 chimeric polypeptide, the GPCR135 or GPCR142 activates a chain of events that alters the concentration of intracellular signaling molecules (second messenger molecule), such as cyclic AMP (cAMP) and calcium via activating a G-protein, which has a high affinity for GTP. These intracellular signaling molecules in turn alter the physiology and behavior of the cell.

As used herein, "GPCR135" and "GPCR142" each refers to a polypeptide that: (1) has greater than about 60% sequence identity to the human receptor protein (GPCR135 or GPCR142, as the case may be); (2) is capable of binding to antibodies, e.g., polyclonal or monoclonal antibodies, raised against the human protein; or (3) is encoded by a polynucleotide that specifically hybridizes under stringent hybridization conditions to a nucleic acid molecule having a sequence that has greater than about 60% nucleotide sequence identity to the human receptor cDNA.

A "receptor component" refers to either GPCR135 or a GPCR142, or an active fragment of either a GPCR135 or a GPCR142.

In preferred embodiments, the receptor component is a biologically active polypeptide having greater than 65,

70, 75, 80, 85, 90, or 95 percent, even more preferably
having 100%, amino acid sequence identity either to human
GPCR135 or human GPCR142. In other preferred
embodiments, the receptor component is a polypeptide
encoded by a polynucleotide that specifically hybridizes
under stringent hybridization conditions to a nucleic
acid molecule having a sequence that has greater than 65,
70, 75, 80, 85, 90, or 95 percent nucleotide sequence
identity to human GPCR135 cDNA or human GPCR142 cDNA.

GPCR135 is also called SALPR (Matsumoto et al.,
2000, *Gene*, 248:183-189; GenBank Protein Accession No.:
BAA93001; GenBank nucleotide Accession No.: D88437 for
cDNA). Exemplary GPCR135 molecules include orthologs
that have been identified in human (SEQ ID NO:25, GenBank
Accession Numbers: BAA93001, Q9NSD7, and NP_057652), rat
(SEQ ID NO:46, and SEQ ID NO:47, 85.9% sequence identity
to that of human), mouse (SEQ ID NO:45, 86.4% sequence
identity to that of human), and other animals, including
pig and monkey. An active fragment of a receptor GPCR135
means any fragment of the receptor protein that maintains
its biological activity, such as binding to a mammalian
relaxin3 and activating a G-protein. The protein or cDNA
sequence of human GPCR135 has been disclosed in WIPO
Publication Nos. WO00/23111, WO00/24891, WO01/48189,
WO01/62797, WO01/74904, and WO01/85791.

Preferred GPCR142 orthologs include human GPCR142
(SEQ ID NO:27), mouse GPCR142 (SEQ ID NO:26), monkey
GPCR142(SEQ ID NO:29), bovine GPCR142 (SEQ ID NO:30), and
porcine GPCR142 (SEQ ID NO:31). An active fragment of
GPCR142 means a polypeptide that maintains the biological
activity of a GPCR142, such as binding to a mammalian
relaxin3 and activating a G-protein. The cDNA or protein
sequence of human GPCR142 is provided in U.S. Patent No.

5,955,309 and WIPO Publication Nos. WO 01/36471, WO
02/61087, and WO 02/00719.

In preferred embodiments, the relaxin-3 chimeric
polypeptides are isolated or purified. An "isolated" or
"purified" polypeptide is intended to refer to one that
is substantially free of cellular material or protein
contaminants from the cell or tissue source from which
the polypeptide is produced and isolated, or
substantially free of chemical precursors or other
chemicals when the polypeptide is chemically synthesized.
For example, polypeptide that is substantially free of
cellular material can include preparations containing
less than about 30%, or preferably 20%, or more
preferably 10%, or even more preferably 5% (by dry
weight) of contaminating proteins. When the polypeptide
is recombinantly produced, it is also preferably
substantially free of culture medium, e.g., culture
medium representing less than about 20%, or more
preferably 10%, or even more preferably 5 % of the volume
of the protein preparation. When the protein is produced
by chemical synthesis, it is preferably substantially
free of chemical precursors or other chemicals, i.e., it
is separated from chemical precursors or other chemicals
that are involved in the synthesis of the protein.
Accordingly such preparations of the polypeptide or
protein have less than about 30%, or preferably 20%, or
more preferably 10%, or even more preferably 5% (by dry
weight) of chemical precursors or compounds other than
the polypeptide of interest.

Biologically active polypeptides can have several
different physical forms. The polypeptide can exist as a
full-length nascent or unprocessed polypeptide, or as
partially processed polypeptides or combinations of
processed polypeptides. The full-length nascent

polypeptide can be post-translationally modified by
specific proteolytic cleavage events that result in the
formation of fragments of the full-length nascent
polypeptide. A fragment, or physical association of
fragments, can have the biological activity associated
with the full-length polypeptide; of course, the degree
of biological activity associated with individual
fragments can vary.

An "active fragment" of a specified polypeptide
means a fragment or derivative of such polypeptide that
maintains biological activity of the polypeptide. For
example, active fragments may be obtained by making
appropriate deletions, substitutions or additions to a
full-length protein that result in a modified sequence
that maintains the desired biological activity and
possesses conserved motifs. Biological activity of,
e.g., a relaxin-3/INSL5 chimeric polypeptide active
fragment can be evidenced, for example, by an ability to
selectively bind to and stimulate a GPCR135 or a GPCR142,
while not binding or stimulating to an LGR7 or an LGR8.

It is within the purview of one of ordinary skill in
the art to determine which amino acid residues or
nucleotides may be added, deleted, or replaced in the
full-length polypeptide or polynucleotide encoding it
without abolishing biological activities of interest,
such as the ability of the inventive relaxin-3 chimeric
polypeptides to selectively bind to GPCR135 or GPCR142,
in order to obtain suitable fragments or derivatives.
For example, the sequence of the particular polypeptide
fragment or derivative may be compared with that of
similar peptides to determine residues or regions of
homology that need to be conserved to maintain activity.

Suitable amino acid substitutions may be determined by replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., by making conservative amino acid replacements. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Suitable variations may be routinely determined by experimentally making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers both to short chains, which are also referred to in the art as, e.g., peptides, oligopeptides and oligomers, and to longer chains, which are often referred to in the art as proteins, of which there are many types. The term also refers to various forms of protein, including the pro, pre-pro, and mature forms, as discussed above.

It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal

amino acids, can be modified in a given polypeptide,
either by natural processes, such as processing and other
post-translational modifications, and by chemical
modification techniques known in the art. Common
modifications that occur naturally in polypeptides are
described in basic texts and in more detailed monographs,
as well as in research literature, and are therefore
within the purview of persons of ordinary skill in the
art. Among the known modifications which can be present
in polypeptides of the present invention are, to name an
illustrative few, acetylation, acylation, ADP-
ribosylation, amidation, covalent attachment of flavin,
covalent attachment of a heme moiety, covalent attachment
of a nucleotide or nucleotide derivative, covalent
attachment of a lipid or lipid derivative, covalent
attachment of phosphotidylinositol, cross-linking,
cyclization, disulfide bond formation, demethylation,
formation of covalent cross-links, formation of cystine,
formation of pyroglutamate, formylation, gamma-
carboxylation, glycosylation, GPI anchor formation,
hydroxylation, iodination, methylation, myristoylation,
oxidation, proteolytic processing, phosphorylation,
prenylation, racemization, selenoylation, sulfation,
transfer-RNA mediated addition of amino acids to proteins
such as arginylation, and ubiquitination.

Several common modifications, such as glycosylation,
lipid attachment, sulfation, gamma-carboxylation of
glutamic acid residues, hydroxylation and ADP-
ribosylation, are described in many basic texts,
including PROTEINS--STRUCTURE AND MOLECULAR PROPERTIES,
2nd ed., T. E. Creighton, W. H. Freeman and Company, New
York (1993). Many reviews are also available on this
subject, such as those provided by Wold,
"Posttranslational Protein Modifications: Perspectives

and Prospects," pgs. 1-12 in <u>POSTTRANSLATIONAL COVALENT</u> <u>MODIFICATION OF PROTEINS</u>, Johnson (ed.), Academic Press, New York (1983); Seifter et al. (1990), *Meth. Enzymol.,* 182, 626-646; and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging" (1992), Ann. N.Y. Acad. Sci., 663, 48-62.

It will be appreciated, as is known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides can be post-translationally modified, including via natural processing or through human manipulation. Circular, branched and branched-circular polypeptides can be synthesized by non-translation natural processes and by entirely synthetic methods as well. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. For example, blockage of the amino or carboxyl group or both in a polypeptide by a covalent modification is common in naturally occurring and synthetic polypeptides, and such modifications can be present in polypeptides of the present invention. For instance, the amino terminal residue of polypeptides made in E. coli or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the peptide, a methionine residue at the NH_2-terminus can be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionineless amino terminal variants of the protein.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell posttranslational

modification capacity and the modification signals
present in the polypeptide amino acid sequence. For
instance, as is known, glycosylation often does not occur
in bacterial hosts such as, for example, E. coli.
Accordingly, when glycosylation is desired, a polypeptide
should be expressed in a glycosylating host, generally a
eukaryotic cell. Insect cells often carry out the same
posttranslational glycosylations as mammalian cells and,
for this reason, insect-cell expression systems have been
developed to express efficiently mammalian proteins
having native patterns of glycosylation, among other
things. Similar considerations apply to other
modifications. It will be appreciated that the same type
of modification can be present in the same or varying
degree at several sites in a given polypeptide. Also, a
given polypeptide can contain many types of
modifications. In general, as used herein, the term
"polypeptide" encompasses all such modifications,
including those that are present in polypeptides
synthesized recombinantly by expressing a polynucleotide
in a host cell.

In preferred embodiments, the relaxin-3 chimeric
polypeptides are polypeptides in which a relaxin-3 chain
B is bonded to a relaxin-3/insulin family member chain A
that is not a relaxin-3 chain A. Isolated
polynucleotides encoding pre-propolypeptides, from which
the preferred relaxin-3 chain-B:relaxin/insulin family
member chain A chimeric polypeptides are derived, are
also provided.

"Polynucleotide" refers to a linear polymer of at
least 2 nucleic acid molecules joined together by
phosphodiester bonds, and may comprise ribonucleotides or
deoxyribonucleotides.

The term "nucleic acid" as used herein refers to a molecule comprised of one or more nucleotides, i.e., ribonucleotides, deoxyribonucleotides, or both. The term includes monomers and polymers of ribonucleotides and deoxyribonucleotides, with the ribonucleotides and/or deoxyribonucleotides being bound together, in the case of the polymers, via 5' to 3' linkages. The ribonucleotide and deoxyribonucleotide polymers may be single- or double-stranded. However, linkages may include any of the linkages known in the art, including, for example, nucleic acids comprising 5' to 3' linkages. The nucleotides may be naturally occurring or may be synthetically produced analogs that are capable of forming base-pair relationships with naturally occurring base pairs. Examples of non-naturally occurring bases that are capable of forming base-pairing relationships include aza and deaza pyrimidine analogs, aza and deaza purine analogs, and other heterocyclic base analogs, wherein one or more of the carbon and nitrogen atoms of the pyrimidine rings have been substituted by heteroatoms, e.g., oxygen, sulfur, selenium, phosphorus, and the like. Furthermore, the term "nucleic acid sequences" contemplates the complementary sequence and includes any nucleic acid sequence that is substantially homologous to the both the nucleic acid sequence and its complement.

In preferred embodiments of the polynucleotides, the molecules are isolated. An "isolated" nucleic acid molecule or polynucleotide is one that is substantially separated from nucleic acid molecules or polynucleotides with differing nucleic acid sequences. Embodiments of the isolated nucleic acid molecule of the invention include cDNA and genomic DNA and RNA, preferably of rat origin, more preferably of human origin.

"Genetic variant" or "variant" means a specific genetic variant which is present at a particular genetic locus in at least one individual in a population and that differs from the wild type.

"Stringent hybridization conditions" has the meaning known in the art. An extensive guide to the hybridization of nucleic acids is found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, and (1989) Tijssen, Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Stringent hybridization conditions may be suitably selected in view of the particular sequence.

Longer sequences hybridize specifically at higher temperatures. Exemplary stringent conditions include a temperature about 5-10 °C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Exemplary stringent conditions further include a salt concentration less than about 1.0 M sodium ion, e.g., about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and a temperature of at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also include the addition of destabilizing agents such as formamide. For selective or

specific hybridization, an exemplary positive signal is at least two times background, optionally 10 times background hybridization. Illustrative stringent hybridization conditions can be as following: 50% formamide, 5×SSC, and 1% SDS, incubating at 42° C, or, 5×SSC, 1% SDS, incubating at 65° C, with wash in 0.2×SSC, and 0.1% SDS at 65° C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes.

As known in the art, alternative splicing sites may be found in intronic or exonic sequences of genes, giving rise to variant polypeptide gene products. Through routine experimentation alternative or cryptic splice junction sites may be found within intronic or exonic sequences or relaxin/insulin family genes, yielding a variety of relaxin/insulin family transcripts, and used to ultimately produce other relaxin-3 chimeric polypeptides within the scope of the invention. It is also known that interspecies and inter-individual polymorphic heterogeneity (genetic variation at a particular genetic locus among individuals in a population) in such intronic or exonic alternative or cryptic splice junction sites can exist. Such heterogeneity can be exploited by the artisan to obtain various relaxin-3 chimeric polypeptides with amino acid sequences of varying length that possess the biological activity associated with the relaxin-3 chimeric polypeptides described supra and infra. Such alternative relaxin-3 chimeric polypeptides and polynucleotides that encode pre-propolypeptides from which such alternative relaxin-3 chimeric polypeptides are derived, are therefore within the scope of the present invention.

Additionally, the inventive polynucleotides encoding alternative relaxin-3 chimeric pre-propolypeptides may be

synthesized or selected by making use of the redundancy
in the genetic code. Various codon substitutions, such as
the silent changes that produce various restriction
sites, may be introduced to optimize cloning into a
plasmid or viral vector or expression in a particular
prokaryotic or eukaryotic system. Mutations in the
polynucleotide sequence may be reflected in the
polypeptide or domains of other peptides added to the
polypeptide to modify the properties of any part of the
encoded polypeptide, to change characteristics such as
affinities for proteins with which the wild-type protein
associates, affinities for antibodies, or
degradation/turnover rates. Where alteration of a
particular function is desired, insertions, deletions or
non-conservative alterations can be engineered to produce
polypeptides with altered functions but retained
biological activity. Such functional alterations can, for
example, alter one or more of the biochemical
characteristics of the polypeptides of the invention so
long as the desired biological activity is maintained.
For example, appropriate alterations can be selected so
as to generate polypeptides that are better suited for
expression, scale up, and the like in the host cells
chosen for expression.

The nucleic acid sequences that encode the pre-
propolypeptides from which the inventive relaxin-3
chimeric polypeptides are derived also may be cloned
using techniques such as those described in the examples
below. Any of a variety of procedures known in the art
can be used to prepare the nucleic acid molecules of the
invention. For example, using cDNA or genomic DNA
libraries, or total mRNA from the suitable cells
identified above, as a template, and appropriate

oligonucleotides as primers, the nucleic acid sequences
that encode the polypeptide chains or domains that
comprise the inventive relaxin-3 chimeric polypeptides
can be amplified according to standard PCR amplification
techniques. The nucleic acid sequences so amplified from
PCR can be used to construct the inventive
polynucleotides that encode the disclosed relaxin-3
chimeric pre-propolypeptides. The so constructed
polynucleotides that encode the disclosed pre-
propolypeptides can then be cloned into an appropriate
vector and characterized by DNA sequence analysis.
Primers can be prepared by standard synthetic techniques,
e.g., using an automated DNA synthesizer.

Another method to isolate a nucleic acid sequence of
the invention is to probe a genomic or cDNA library, or
total mRNA with one or more natural or artificially
designed probes using procedures available to those
skilled in the art. See, e.g., "Current Protocols in
Molecular Biology", Ausubel et al. (eds.), Greene
Publishing Association and John Wiley Interscience, New
York, 1992. Preferred probes will have from 30 to 50
bases. Such probes can be labeled with an analytically
detectable reagent to facilitate identification of the
probe. Useful reagents include, e.g., radioisotopes,
fluorescent dyes, or enzymes capable of catalyzing the
formation of a detectable product. The probes enable the
ordinarily skilled artisan to isolate complementary
copies of genomic DNA, cDNA or RNA polynucleotides
encoding relaxin/insulin family members. The probes also
enable the ordinarily skilled artisan to isolate
complementary copies of genomic DNA, cDNA or RNA
polynucleotides encoding GPRC135, such as from mouse as
well as human, rat, monkey, cow, and pig, and to isolate
complementary copies of genomic DNA, cDNA or RNA

polynucleotides encoding GPRC142, such as from mouse as well as human, monkey, cow, and pig.

Another method to prepare nucleic acid sequences is by standard synthetic techniques, e.g., using an automated DNA synthesizer. Construction of genomic DNA libraries, preparation of cDNA libraries, or isolation of total mRNA from the identified source cell can be performed by standard techniques known in the art. These techniques can be found, for example, in Maniatis et al., "Molecular Cloning: A Laboratory Manual," 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

Further embodiments provide engineered polynucleotides having any such nucleotide variations that are not known to occur naturally which encode polypeptides having properties that are different than, but still maintain biological activity of the non-variant relaxin-3 chimeric polypeptides. DNA sequences can be altered manually so as to code for a relaxin-3/insulin family member chain A, chain B, or chain C peptide having properties that are different from those of the naturally occurring chain A, chain B, or chain C peptides. Known methods of altering the DNA sequences include site-directed mutagenesis, chimeric substitution, and gene fusions. Site-directed mutagenesis may be used to change one or more DNA residues that can result in a silent mutation, a conservative mutation, or a nonconservative mutation. Chimeric genes may be prepared by swapping chains or domains of similar or different relaxin-3/insulin family member genes to replace such chains or domains in the human relaxin-3/insulin family member gene. Similarly, fusion genes can be prepared that add domains to a relaxin-3/insulin family member gene, such

as an affinity tag to facilitate identification and isolation of the gene and protein.

The biological activity of relaxin-3 chimeric polypeptides disclosed herein as well as, e.g., polymorphic variants, interspecies variants, as well as interspecies homologs and alleles, and engineered variants, can be confirmed by examining, e.g., selectivity of binding and stimulating a GPCR135 or a GPCR142, while not binding to or stimulating an LGR7 or an LGR8, of the putative relaxin-3 chimeric polypeptide variant. For example, the biological activity of the relaxin-3/INSL5 chimeric polypeptide having an amino acid sequence of SEQ ID NO:23 can be used as a positive control in comparison to other relaxin-3/chimeric polypeptide variants to confirm conservation of biological activity.

In a preferred embodiment, the relaxin-3 chimeric polypeptide is a biologically active polypeptide having at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:23. In an especially preferred embodiment, the relaxin-3 chimeric polypeptide has a sequence as set forth in SEQ ID NO:23.

"Sequence identity or similarity or homology", as known in the art, is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences.

To determine the percent identity or similarity of two amino acid sequences or of two nucleic acids, the

sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same or similar amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical or similar at that position. The percent identity or similarity between the two sequences is a function of the number of identical or similar positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100).

Both identity and similarity can be readily calculated. In calculating percent identity, only exact matches are counted. Methods commonly employed to determine identity or similarity between sequences include, e.g., those disclosed in Carillo et al., 1988, *SIAM J. Applied Math.*, 48:1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Exemplary methods to determine identity and similarity are also provided in commercial computer programs. A preferred example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:2264-2268, modified as in Karlin et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, *J. Mol. Biol.*, 215:403-410. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.*, 25:3389-3402.

Alternatively, PSI-Blast can be used to perform an iterated search, which detects distant relationships between molecules. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See, e.g., http://www.ncbi.nlm.nih.gov. Additionally, the FASTA method (Atschul et al., 1990, *J. Mol. Biol.*, 215:403) can be used. Another preferred example of a mathematical algorithm useful for the comparison of sequences is the algorithm of Myers et al., 1988, *CABIOS*, 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package (Devereux et al., 1984, *Nucleic Acids Res.*, 12(1):387).

In various embodiments, the inventive polynucleotides are inserted into expression vectors for introduction of such polynucleotides into host cells for the expression, i.e., production of the encoded mRNA or protein, of the relaxin-3 chimeric pre-propolypeptides encoded by such polynucleotides in such host cells. In certain embodiments, chimeric polypeptides of the invention are produced by recombinant DNA techniques. The expressed relaxin-3 chimeric pre-propolypeptides are processed the corresponding mature forms in vivo, and the resulting recombinant host cells are isolated for various uses in vitro, or serve to modulate various other in vivo activities within such recombinant host cells.

A host cell, which is a cell that contains a DNA molecule either in a vector or integrated into a cell chromosome, can be either a native host cell that contains an endogenous DNA molecule, or a recombinant host cell. Exemplary host cells include bacterial cells,

yeast cells, and animal cells. Preferred host cells
include mammalian cells, more preferably human cells.

    The term "cell" refers to at least one cell or a
plurality of cells appropriate for the sensitivity of the
detection method. Cells suitable for the present
invention may be bacterial, but are preferably
eukaryotic, such as yeast, insect, or mammalian. The
cell can be a natural host cell for an endogenous GPCR135
or GPCR142, or both GPCR135 and GPCR142, or preferably a
recombinant host cell for a GPCR135 or GPCR142, or both
GPCR135 and GPCR142, which expresses increased amount of
a mammalian GPCR135 or GPCR142, or both GPCR135 and
GPCR142, on the cell surface.

    A "recombinant host cell" is a cell that has been
transformed or transfected by an exogenous DNA sequence.
As used herein, a cell has been "transformed" by
exogenous DNA when such exogenous DNA has been introduced
inside the cell membrane. Exogenous DNA may or may not
be integrated (covalently linked) into chromosomal DNA
making up the genome of the cell. In prokaryotes and
yeasts, for example, the exogenous DNA may be maintained
on an episomal element, such as a plasmid. With respect
to eukaryotic cells, a stably transformed or transfected
cell is one in which the exogenous DNA has become
integrated into the chromosome so that it is inherited by
daughter cells through chromosome replication. This
stability is demonstrated by the ability of the
eukaryotic cell to establish cell lines or clones
comprised of a population of daughter cells containing
the exogenous DNA. Recombinant host cells may be
prokaryotic or eukaryotic, including bacteria such as E.
coli, fungal cells such as yeast, mammalian cells such as
cell lines of human, bovine, porcine, monkey and rodent

origin, and insect cells such as Drosophila and silkworm derived cell lines. It is further understood that the term "recombinant host cell" refers not only to the particular subject cell, but also to the progeny or potential progeny of such a cell. Because certain modifications can occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term.

A clone is a population of cells derived from a single cell or common ancestor by mitosis. A cell line is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

In another aspect, the present invention provides vectors, preferably expression vectors, containing a nucleic acid that is capable of expressing the inventive polynucleotides encoding the disclosed relaxin-3 chimeric pre-propolypeptides. In a preferred embodiment, the preferred expression vector contains a polynucleotide encoding the inventive relaxin-3/INSL5 chimeric pre-propolypeptide.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double-stranded DNA loop into which additional DNA segments can be inserted. Another type of vector is a viral vector wherein additional DNA segments can be inserted. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e. g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon

introduction into the host cell, and thereby are
replicated along with the host genome. Moreover, certain
vectors--expression vectors--are capable of directing the
expression of genes to which they are operably linked.
Vectors of utility in recombinant DNA techniques may be
in the form of plasmids. Alternatively, other forms of
vectors, such as viral vectors (e.g., replication
defective retroviruses, adenoviruses and adeno-associated
viruses), which serve equivalent functions, may be used.

     The recombinant expression vectors of the invention
comprise a nucleic acid of the invention in a form
suitable for expression of the nucleic acid in a host
cell. Thus, the recombinant expression vectors can
include one or more regulatory sequences, such as a
promoter, selected on the basis of the host cells to be
used for expression, operably linked to the nucleic acid
sequence to be expressed. When used in reference to a
recombinant expression vector, "operably linked" is
intended to mean that the nucleotide sequence of interest
is linked to the regulatory sequence(s) in a manner
allowing for expression of the nucleotide sequence (e.g.,
in an *in vitro* transcription/translation system or in a
host cell when the vector is introduced into the host
cell). It will be appreciated by those of ordinary skill
in the art that the design of the expression vector can
depend on such factors as the choice of the host cell to
be transformed and the level of expression of protein
desired as well as the intended use of the vector. The
expression vectors of the invention can be introduced
into host cells to thereby produce proteins or peptides,
including fusion proteins or peptides, encoded by nucleic
acids as described herein. One exemplary application of
vectors according to the invention is use in therapeutic
treatment methods as described below.

"Promoter" means a regulatory sequence of DNA that is involved in the binding of RNA polymerase to initiate transcription of a gene. Promoters are often upstream (i.e., 5' to 3') the transcription initiation site of the gene. A "gene" is a segment of DNA involved in producing a peptide, polypeptide, or protein, including the coding region, non-coding regions preceding ("5'UTR") and following ("3'UTR") coding region, as well as intervening non-coding sequences ("introns") between individual coding segments ("exons"). "Coding" refers to the specification of particular amino acids or termination signals in three-base triplets ("codons") of DNA or mRNA.

The recombinant expression vectors of the invention can be designed for expression of a polypeptide of the invention in prokaryotic (e.g., Escherichia coli (E. coli)) or eukaryotic cells (e.g., insect cells (using baculovirus expression vectors), yeast cells or mammalian cells). Suitable host cells may be routinely determined. Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes may be carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically are used for one or more of the following purposes: to increase expression of recombinant protein; to increase the solubility of the recombinant protein; to aid in the purification of the recombinant protein by acting as a ligand in affinity purification; and to facilitate detection of the recombinant protein by serving as a marker. Often in fusion expression vectors,

a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes and their cognate recognition sequences include Factor Xa, thrombin and enterokinase. Exemplary fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith et al., 1988, *Gene*, 67:31-40), pMAL (New England Biolabs, Beverly, MA), pRIT5 (Pharmacia, Piscataway, NJ), or pQE (Qiagen), which fuse glutathione S-transferase (GST), maltose binding protein, protein A, or poly-His, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., 1988, *Gene*, 69:301-315) and pETIId (Studier et al., "Gene Expression Technology: Methods in Enzymology 185," Academic Press, San Diego, California, 1990, 60-89). One strategy to maximize recombinant protein expression in E. coli is to express the protein in a host bacterium with an impaired capacity to proteolytically cleave the recombinant protein. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in E. coli. Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another preferred embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in yeast S. cerivisae include pYepSec1 (Baldari et al., 1987, *EMBO J*, 6:229-234), pMFa (KurJan et al., 1982, *Cell*, 30:933-943), pJRY88 (Schultz et al. 1987, *Gene*, 54:113-123), pYES2 (Invitrogen Corporation,

San Diego, CA), and pPicZ or Pichia (Invitrogen Corp, San Diego, CA).

Alternatively, the expression vector is a baculovirus expression vector. Baculovirus vectors available for expression of proteins in cultured insect cells include, e.g., the pAc series (Smith et al., 1983, *Mol. Cell. Biol.*, 3:2156-2165) and the pVL series (Lucklow et al., 1989, *Virology*, 170:31-39). Commercially available insect cell expression vectors useful for recombinant expression include pBlueBacII (Invitrogen).

In yet another preferred embodiment, the expression vector is a mammalian expression vector. When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. Examples of mammalian expression vectors include, e.g., pCDM8 (Seed, 1987, *Nature*, 329:840) and pMT2PC (Kaufinan et al., 1987, *EMBO J*, 6:187-195). Commercially available mammalian expression vectors which can be suitable for recombinant protease COX-3 expression include, for example, pMAMneo (Clontech), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and 1ZD35 (ATCC 37565).

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Examples of suitable

tissue-specific promoters include the albumin promoter
(liver-specific; Pinkert et al., 1987, *Genes Dev.*, 1:268-
277), lymphoid-specific promoters (Calame et al., 1988,
Adv. Immunol., 43:235-275), in particular promoters of T
cell receptors (Winoto et al., 1989, *EMBO J*, 8:729-733)
and immunoglobulins (BaneiJi et al., 1983, *Cell*, 33:729-
740; Queen et al., 1983, *Cell*, 33:741-748), neuron-
specific promoters (e.g., the neurofilament promoter;
Byme et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:5473-
5477), pancreas-specific promoters (Edlund et al., 1985,
Science, 230:912-916), and mammary gland-specific
promoters (e.g., milk whey promoter; U.S. Patent No.
4,873,316 and European Patent Publication No. 264,166).
Developmentally regulated promoters also include, for
example, the marine hox promoters (Kessel et al., 1990,
Science, 249:374-379) and the beta-fetoprotein promoter
(Campes et al., 1989, *Genes Dev.*, 3:537-546).

Some expression systems have markers that provide
gene amplification, such as neomycin, thymidine kinase,
hygromycin B phosphotransferase, and dihydrofolate
reductase. Alternatively, high yield expression systems
not involving gene amplification are also suitable, such
as using a baculovirus vector in insect cells, with a
sequence encoding the polypeptide under the direction of
the polyhedrin promoter or other strong baculovirus
promoters.

The elements that can be included in expression
vectors also include a replicon that functions in *E.
coli*, a gene encoding antibiotic resistance to permit
selection of bacteria that harbor recombinant plasmids,
and unique restriction sites in nonessential regions of
the plasmid to allow insertion of eukaryotic sequences.
The particular antibiotic resistance gene chosen is not

critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences may be chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary or desired.

A recombinant vector may be prepared comprising a DNA molecule of the invention cloned into the vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to the mRNA encoding a polypeptide of the invention. Regulatory sequences operably linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue-specific or cell-type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high-efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. ("Reviews - Trends in Genetics," Vol. 1(1) 1986).

A recombinant vector system may also be prepared to direct the synthesis of small interfering RNAs (siRNAs) in mammalian cells. Many organisms possess mechanisms to silence any gene expression when double-stranded RNA (dsRNA) corresponding to the gene is present in the cell through a process known as RNA interference. The technique of using dsRNA to reduce the activity of a

specific gene was first developed using the worm C.
elegans and has been termed RNA interference or RNAi
(Fire et al., 1998, *Nature*, 391: 806-811). RNAi has
since been found to be useful in many organisms, and
recently has been extended to mammalian cells in culture
(see review by Moss, 2001, *Curr Biol*, 11:R772-R775). An
important advance was made when RNAi was shown to involve
the generation of small RNAs of 21-25 nucleotides
(Hammond et al., 2000, *Nature*, 404:293-296; Zamore et
al., 2000, *Cell*, 101:25-33). These small interfering
RNAs, or siRNAs, may initially be derived from a larger
dsRNA that begins the process, and are complementary to
the target RNA that is eventually degraded. The siRNAs
are themselves double-stranded with short overhangs at
each end; they act as guide RNAs, directing a single
cleavage of the target in the region of complementarity
(Elbashir et al., 2001, *Genes Dev.*, 15:188-200; Zamore et
al., 2000, *Cell*, 101: 25-33).

An siRNA comprising nucleotides that are
complementary to mouse GPCR135 or GPCR142 may be produced
in vitro, for example, using a method described in WIPO
Publication No. WO 01/75164, or can be produced *in vivo*
from a mammalian cell using a stable expression system.
An exemplary vector system that directs the synthesis of
siRNAs in mammalian cells is the pSUPER (Brummelkamp et
al., 2002, *Science*, 296: 550-553).

Exemplary vectors of the present invention also
include specifically designed vectors that allow the
shuttling of DNA between hosts such as bacteria-yeast or
bacteria-animal cells or bacteria-fungal cells or
bacteria-invertebrate cells. Numerous cloning vectors
are known to those skilled in the art and the selection
of an appropriate cloning vector is within the purview of
the artisan. For other suitable expression systems for

both prokaryotic and eukaryotic cells see, e.g., chapters 16 and 17 of Maniatis et al., supra.

Recombinant host cells may be prepared by introducing a recombinant vector of the invention. Cell lines derived from mammalian species which can be suitable for transfection and which are commercially available include, e.g., CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), Drosophila or murine L-cells, and HEK-293 (ATCC CRL1573), and monkey kidney cells.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the term "transformation" or "transfection" refers to a process by which cells take up foreign DNA and may or may not integrate that foreign DNA into their chromosome. Any of the known procedures suitable for introducing foreign nucleotide sequences into host cells may be used to introduce the expression vector. These include the use of reagents such as Superfect (Qiagen), liposomes, calcium phosphate transfection, polybrene, protoplast fusion, electroporation, microinjection, plasmid vectors, viral vectors, biolistic particle acceleration (the Gene Gun), or any other known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, e. g., Sambrook et al., supra). The selected particular genetic engineering procedure used should be capable of successfully introducing at least one gene into the host cell capable of expressing the mRNA, cDNA, or gene.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) may be introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

In another embodiment, the expression characteristics of an endogenous nucleic acid within a cell, cell line or microorganism can be modified by inserting a heterologous regulatory element into the genome of a cell, a stable cell line, or a cloned microorganism, such that the inserted regulatory element is operatively linked with the endogenous gene and controls, modulates or activates the endogenous gene. A heterologous regulatory element can be inserted into a stable cell line or cloned microorganism such that it is operatively linked with and activates expression of endogenous genes, using techniques such as targeted homologous recombination, e.g., as described in U.S. Patent No. 5,272,071 and WIPO Publication No. WO 91/06667.

Polypeptides of the invention can be recombinantly expressed by cloning DNA molecules of the invention into an expression vector described above, introducing such a vector into prokaryotic or eukaryotic host cells as described herein, and growing the host cell under

conditions suitable for production of recombinant protein.

The expression vector-containing cells may be clonally propagated and individually analyzed to determine whether they produce the polypeptide of the invention. Identification of the animal relaxin-3 chimeric polypeptide-expressing host cell clones can be done by several means, including immunological reactivity with anti-animal relaxin-3 chimeric polypeptide antibodies, and the presence of host cell-associated relaxin-3 chimeric polypeptide, such as GPCR135 or GPCR142 binding or stimulation. The selection of the appropriate growth conditions and recovery methods are within the skill of the art. Techniques for recombinantly expressing a polypeptide are described in, e.g., Maniatis et al., supra.

Polypeptides of the invention can also be produced using an *in vitro* translation and/or transcription system available in the art. For example, synthetic relaxin-3 chimeric pre-propolypeptide mRNA or relaxin-3 chimeric pre-propolypeptide mRNA isolated from transformed or transfected cells can be efficiently translated in various cell-free systems, including wheat germ extracts and reticulocyte extracts. Alternatively, the coding sequence of the inventive relaxin-3 chimeric pre-propolypeptide cDNA can be cloned under the control of a T7 promoter. Then, using this construct as the template, the protein can be produced in an *in vitro* transcription and translation system, for example using a TNT T7 coupled Reticulocyte Lysate System such as that commercially available from Promega (Madison, WI).

In a general aspect, the invention is directed to receptor-ligand complexes comprising a relaxin-3 chimeric polypeptide component and a GPCR135 or GPCR142 receptor

component. In some embodiments of the invention, ligand-and-receptor complexes are detected, identified, formed, or isolated.

The components of the complex (e.g., relaxin-3 chimeric polypeptides along with GPCR135 and/or GPCR142 polypeptides) can be purified by methods known to those skilled in the art. For example, they can be purified from cell lysates and extracts from natural or recombinant host cells, by various combinations or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography, lectin chromatography, HPLC, and FPLC, and antibody/ligand affinity chromatography. Further alternatively, a polypeptide of the invention can be synthesized chemically using standard peptide synthesis techniques, and purified as described supra.

The isolated and purified relaxin-3 chimeric polypeptides of the invention may be used in assay methods for selectively detecting, identifying, or stimulating or GPCR135 or GPCR142 (i.e., while not detecting, identifying, or stimulating LGR7 or LGR8) in test biological samples, in methods for identifying compounds having GPCR135 or GPCR142 modulating activities, and in treating medical conditions mediated by modulation of GPCR135 or GPCR142.

In certain embodiments of such methods, naturally occurring GPCR135 or GPCR142-containing biological samples are isolated, e.g., from mammalian tissue such as brain, spleen, placenta, lung, liver, kidney, pancreas, prostate, testis, ovary, small intestine, colon, lymph node, and tonsils, or any other source of a GPCR135 or GPCR142 homolog. Bodily fluids such as blood, blood

plasma, serum, seminal fluid, urine, or any other mammalian bodily fluid can also serve as sources of natural GPCR135 or GPCR142 polypeptides. Cultured mammalian cell lines are still further exemplary sources of natural GPCR135- or GPCR142-containing biological samples.

In especially preferred embodiments, the GPCR135 or GPCR142-containing biological sample is derived from mammalian brain tissue, preferably rat brain tissue, and more preferably from human brain tissue. In still other preferred embodiments, the GPCR135 or GPCR142- containing biological sample is derived, from human cell lines, such as G292 (ATCC # CRL-1423), HeLa (ATCC # CCL-2), Jurkat (ATCC # TIB-152),and THP-1 (ATCC # TIB-20). In still further preferred embodiments, polypeptides are derived from monocytes substantially purified from peripheral blood and from monocyte-derived dendritic cells. Methods of purification of monocytes substantially purified from peripheral blood are known in the art; for example, such cells may be adsorbed onto CD14 microbeads and subsequently separated on an Auto Macs (Miltenyi Biotech). An exemplary method to derive dendritic cells from monocytes is to culture the monocytes in the presence of GM-CSF and IL-4 in endotoxin-free media and reagents.

A "ligand component" refers to a relaxin-3 chimeric polypeptide in accordance with the invention, such as those described above. In a preferred embodiment, the ligand component is composed of a relaxin-3 chain B that is originated from a human, a mouse, or a rat, that is bonded via a disulfide linkage to a relaxin/insulin family member chain A that is originated from a human. Preferably, the ligand component has been recombinantly

expressed.   Preferred ligand components correspond to the
relaxin-3 chimeric polypeptides identified in the
accompanying Sequence Listing.

In other preferred embodiments of the ligand-and-
receptor complexes of the invention, the ligand component
is labeled with a detectable agent, such as a
radioisotope or a fluorescent molecule.   The labeling
technique is selected based on the type of labeling agent
employed, and is within the purview of those ordinarily
skilled in the art.   For instance, labeling can be
accomplished by replacing one of the atoms of a ligand
molecule with a corresponding radioactive isotope.   A
hydrogen atom could be replaced with tritium, ^{3}H; a carbon
atom can be replaced with carbon-14, ^{14}C; or a strontium
atom can be replaced with strontium-38, ^{38}Sr.   In another
exemplary labeling process, rather than replacing any
atoms of the ligand with a radioactive isotope, an
isotope can be added to the ligand molecule. Such
radioactive isotopes include, for example, iodine-125,
^{125}I, and iron-59, ^{59}Fe.   In yet another exemplary labeling
process, labeling can be carried out by using an
appropriate radiolabeled precursor, such as methionine-35
(^{35}S) or phosphate-33 (^{33}P, for protein phosphorylation),
during the synthesis of the peptide either *in vivo* or *in
vitro*.   Preferably, the ligand component of this
invention is labeled with iodine-125, ^{125}I.

The receptor component of the complex is preferably
a mammalian GPCR135 or a mammalian GPCR142. More
preferably, the receptor of the complex is a human
GPCR135 or a human GPCR142.

In a certain preferred embodiment, the GPCR135 or
GPCR 142, or both GPCR135 and GPCR 142, is expressed on
the cell surface of a host cell, preferably a recombinant
host cell.   In another preferred embodiment, the GPCR135

or GPCR142, or both GPCR135 and GPCR 142, is associated with isolated cell membranes from a host cell, preferably from a recombinant recombinant host cell. In yet another preferred embodiment, the receptor component of the complex is a fragment of the GPCR135 or a fragment of the GPCR142 capable of binding to an inventive relaxin-3 chimeric polypeptide ligand. Any suitable methods for constituting a ligand component and receptor complex available to artisans may be used to form such a complex. For example, such a method generally comprises mixing a sample comprising the ligand component with a sample comprising the receptor.

The sample comprising the ligand component can be tissue or cell extract containing the ligand component, or purified ligand component. This sample can be prepared from a natural source of the ligand component, e.g., an endogenous host cell or tissue for the ligand component of warm-blooded animals inclusive of human. Preferably, the sample comprising the ligand component is prepared from a recombinant host cell that expresses increased amount of the ligand component. A recombinant host cell for the ligand component may be constructed by introducing into the cell a DNA molecule capable of expressing the functional ligand component.

In the production from the tissues or cells of human or other warm-blooded animals, the ligand component chimerae can be purified and isolated by a process comprising homogenizing the tissue or cells of human or other warm-blooded animal, extracting the homogenate with an acid or another suitable extracting agent, and isolating the ligand component polypeptide from the extract.

Any of a number of suitable procedures can be employed when the inventive relaxin-3 chimeric polypeptides or a GPCR135 or GPCR142 are being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to the polypeptides. With the appropriate ligand, the polypeptide can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. The polypeptides can also be purified using immunoaffinity columns.

Recombinant proteins may be expressed by transformed bacteria or eukaryotic cells in large amounts, preferably after promoter induction; but expression can be constitutive. Promoter induction with IPTG is one example of an inducible promoter system. Cells may be grown according to standard procedures in the art. Fresh or frozen cells may be used for isolation of protein.

Proteins expressed in bacteria may form insoluble aggregates (inclusion bodies). Several protocols described in the art are suitable for purification of relaxin-3 chimeric polypeptide-, GPCR135-, or GPCR142-containing inclusion bodies. For example, purification of inclusion bodies may involve the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells, e.g., by incubation in a buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM MgCl2, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria will be apparent to those of ordinary skill in the art (see, e.g., Sambrook et al., *supra*; Ausubel et al., *supra*).

If necessary or desired, the inclusion bodies may be solubilized, and the lysed cell suspension centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents which are capable of solubilizing aggregate-forming proteins, for example SDS (sodium dodecyl sulfate) and 70% formic acid, may be inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known in the art. The desired polypeptides are separated from other bacterial proteins by standard separation techniques, e.g., with Ni-NTA agarose resin.

Alternatively, it is possible to purify polypeptides from bacteria periplasm. After lysis of the bacteria, when a protein is exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock or another method known in the art. To isolate recombinant proteins from the periplasm, the bacterial cells may be centrifuged to form a pellet. The pellet may be resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria may be centrifuged and the pellet resuspended in ice-cold

5 mM MgSO$_4$ and kept in an ice bath for approximately 10
minutes. The cell suspension may be centrifuged and the
supernatant decanted and saved. The recombinant proteins
present in the supernatant can be separated from the host
proteins by standard separation techniques known in the
art.

As an initial step, e.g., if a protein mixture is
complex, an initial salt fractionation can be used to
separate many of the unwanted host cell proteins (or
proteins derived from the cell culture media) from the
recombinant protein of interest. An exemplary salt is
ammonium sulfate, which precipitates proteins by
effectively reducing the amount of water in the protein
mixture. Proteins then precipitate on the basis of their
solubility. The more hydrophobic a protein is, the more
likely it is to precipitate at lower ammonium sulfate
concentrations. An exemplary isolation protocol includes
adding saturated ammonium sulfate to a protein solution
so that the resultant ammonium sulfate concentration is
between 20-30%. This concentration will precipitate the
most hydrophobic of proteins. The precipitate is then
discarded (unless the protein of interest is hydrophobic)
and ammonium sulfate is added to the supernatant to a
concentration known to precipitate the protein of
interest. The precipitate is then solubilized in buffer
and the excess salt removed to achieve the desired
purity, e.g., through dialysis or diafiltration. Other
known methods that rely on solubility of proteins, such
as cold ethanol precipitation, can be used to fractionate
complex protein mixtures.

In other examples, the molecular weight of the
inventive relaxin-3 chimeric polypeptide, or a GPCR135 or
a GPCR142, can be used to isolate it from proteins of

greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut-off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed.

The inventive chimeric polypeptides can also be separated from other proteins on the basis of net surface charge, hydrophobicity, and affinity for heterologous molecules. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are known in the art. It will be apparent to those of ordinary skill in the art that chromatographic techniques can be performed at any suitable scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

The invention also permits the identification of antibodies that specifically bind to the relaxin-3 chimeric polypeptides. Such relaxin-3 chimeric polypeptide-specific antibodies have numerous applications, including for the modulation of relaxin-3 chimeric polypeptide activity and for immunoassays to detect the relaxin-3 chimeric polypeptides. Immunoassays can be used to qualitatively or quantitatively analyze relaxin-3 chimeric polypeptides. A general overview of such technology can be found in Harlow & Lane, ANTIBODIES: A LABORATORY MANUAL (1988).

Methods of producing polyclonal and monoclonal
antibodies that react specifically with relaxin-3
chimeric polypeptide polypeptides are known in the art
(see, e.g., Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY (1991);
Harlow & Lane, *supra*; Goding, MONOCLONAL ANTIBODIES: PRINCIPLES
AND PRACTICE (2d ed. 1986); and Kohler & Milstein, 1975,
Nature 256:495-497). Such techniques include antibody
preparation by selection of antibodies from libraries of
recombinant antibodies in phage or similar vectors, as
well as preparation of polyclonal and monoclonal
antibodies by immunizing rabbits or mice (see, e.g., Huse
et al., 1989, *Science,* 246:1275-1281; Ward et al., 1989,
Nature, 1989, 341:544-546).

A number of relaxin-3 chimeric polypeptide-
comprising immunogens may be used to produce antibodies
specifically reactive with a relaxin-3 chimeric
polypeptide. For example, a relaxin-3 chimeric
polypeptide, or an antigenic fragment thereof, is
isolated as described herein. Recombinant protein can be
expressed in eukaryotic or prokaryotic cells as described
above, and purified as generally described above.
Recombinant protein is the preferred immunogen for the
production of monoclonal or polyclonal antibodies.
Alternatively, a synthetic peptide derived from the
sequences disclosed herein and conjugated to a carrier
protein can be used an immunogen. Naturally occurring
protein may also be used either in pure or impure form.
The product is then injected into an animal capable of
producing antibodies. Either monoclonal or polyclonal
antibodies may be generated, for subsequent use in
immunoassays to measure the protein.

Methods of production of polyclonal antibodies are
known in the art. In one exemplary method, an inbred

strain of mice (e.g., BALB/C mice) or rabbits is
immunized with the protein using a standard adjuvant,
such as Freund's adjuvant, and a standard immunization
protocol. The animal's immune response to the immunogen
preparation is monitored by taking test bleeds and
determining the titer of reactivity to the polypeptide.
When appropriately high titers of antibody to the
immunogen are obtained, blood is collected from the
animal and antisera are prepared. Further fractionation
of the antisera to enrich for antibodies reactive to the
protein can be done if desired (see, Harlow & Lane,
supra).

Monoclonal antibodies may be obtained by various
techniques described in the art. To illustrate briefly,
spleen cells from an animal immunized with a desired
antigen may be immortalized, e.g., by fusion with a
myeloma cell (see Kohler & Milstein, *Eur. J Immunol.*,
1976, 6:511-519). Alternative methods of immortalization
include transformation with Epstein Barr Virus,
oncogenes, or retroviruses. Colonies arising from single
immortalized cells are screened for production of
antibodies of the desired specificity and affinity for
the antigen, and yield of the monoclonal antibodies
produced by such cells may be enhanced by various
techniques, including injection into the peritoneal
cavity of a vertebrate host. Alternatively, one may
isolate DNA sequences which encode a monoclonal antibody
or a binding fragment thereof by screening a DNA library
from human B cells according to the general protocol
outlined by Huse et al., 1989, *Science*, 246:1275-1281.

Monoclonal antibodies and polyclonal sera may be
collected and titered against the immunogen protein in an
immunoassay, for example, a solid phase immunoassay with

the immunogen immobilized on a solid support. To
illustrate, polyclonal antisera with a titer of 10^4 or
greater are selected and tested for their cross-
reactivity against unrelated proteins, or even related
proteins from other organisms, using a competitive
binding immunoassay. Specific polyclonal antisera and
monoclonal antibodies may bind with a Kd of 1 µM, 0.1 µM
or 0.01 µM or better.

Using anti-relaxin-3 chimeric polypeptide
antibodies, individual relaxin-3 chimeric polypeptides
can be detected by a variety of immunoassay methods. For
a review of immunological and immunoassay procedures, see
BASIC AND CLINICAL IMMUNOLOGY (Stites & Terr eds., 7th ed.
1991). Moreover, immunoassays can be performed in any of
several configurations, which are reviewed extensively in
ENZYME IMMUNOASSAY (Maggio, ed., 1980); and Harlow & Lane,
supra.

Biologically active relaxin-3 chimeric polypeptides
can be detected and/or quantified using any of a number
of known immunological binding assays (see, e.g., U.S.
Patent Nos. 4,366,241; 4,376,110; 4,517,288; and
4,837,168). For a review of the general immunoassays, see
also METHODS IN CELL BIOLOGY: ANTIBODIES IN CELL BIOLOGY, volume
37 (Asai, ed. 1993); BASIC AND CLINICAL IMMUNOLOGY (Stites &
Terr, eds., 7th ed. 1991). Immunological binding assays
(or immunoassays) generally use an antibody that
specifically binds to a protein or antigen of choice (in
this case a relaxin-3 chimeric polypeptide or an
antigenic subsequence thereof). The antibody may be
produced by any suitable means known in the art.

Immunoassays also may employ a labeling agent to
specifically bind to and label the complex formed by the

antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled relaxin-3 chimeric polypeptide or a labeled anti-relaxin-3 chimeric polypeptide antibody. Alternatively, the labeling agent may be a third moiety, such a secondary antibody, that specifically binds to the antibody/relaxin-3 chimeric polypeptide complex (a secondary antibody is preferably specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G, may be used as the label agent. These proteins exhibit a strong nonimmunogenic reactivity with immunoglobulin constant regions from a variety of species (see, e.g., Kronval et al., 1973, *J. Immunol.*, 111:1401-1406; Akerstrom et al., 1985, *J. Immunol.*, 135:2589-2542). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are known in the art.

Throughout the assays, incubation and/or washing steps may be appropriate after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, optionally from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, antigen, volume of solution, concentrations, and the like. The assays may be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

Immunoassays for detecting a relaxin-3 chimeric polypeptide in a sample may be either competitive or

noncompetitive. Noncompetitive immunoassays are assays in
which the amount of antigen is directly measured. In one
preferred sandwich assay, for example, the anti-relaxin-3
chimeric polypeptide antibodies can be bound directly to
a solid substrate on which they are immobilized. These
immobilized antibodies then capture the relaxin-3
chimeric polypeptide present in the test sample. The
relaxin-3 chimeric polypeptide thus immobilized is then
bound by a labeling agent, such as a second relaxin-3
chimeric polypeptide antibody bearing a label.
Alternatively, the second antibody may lack a label, but
it may, in turn, be bound by a labeled third antibody
specific to antibodies of the species from which the
second antibody is derived. The second or third antibody
is often modified with a detectable moiety, such as
biotin, to which another molecule specifically binds,
e.g., streptavidin, to provide a detectable moiety.

In competitive assays, the amount of relaxin-3
chimeric polypeptide present in the sample may be
measured indirectly by measuring the amount of a known,
added (exogenous) relaxin-3 chimeric polypeptide
displaced (competed away) from an anti-relaxin-3 chimeric
polypeptide antibody by the unknown relaxin-3 chimeric
polypeptide present in a sample. In one exemplary
competitive assay, a known amount of relaxin-3 chimeric
polypeptide is added to a sample and the sample is then
contacted with an antibody that specifically binds to the
relaxin-3 chimeric polypeptide. The amount of exogenous
relaxin-3 chimeric polypeptide bound to the antibody is
inversely proportional to the concentration of relaxin-3
chimeric polypeptide present in the sample. In an
exemplary embodiment, the antibody is immobilized on a
solid substrate. The amount of relaxin-3 chimeric

polypeptide bound to the antibody may be determined either by measuring the amount of relaxin-3 chimeric polypeptide present in an relaxin-3 chimeric polypeptide /antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of relaxin-3 chimeric polypeptide may be detected by providing a labeled relaxin-3 chimeric polypeptide molecule.

A hapten inhibition assay is another illustrative competitive assay. In this assay the known relaxin-3 chimeric polypeptide is immobilized on a solid substrate. A known amount of anti relaxin-3 chimeric polypeptide antibody is added to the sample, and the sample is then contacted with the immobilized relaxin-3 chimeric polypeptide. The amount of anti relaxin-3 chimeric polypeptide antibody bound to the known immobilized relaxin-3 chimeric polypeptide is inversely proportional to the amount of relaxin-3 chimeric polypeptide present in the sample. Again, the amount of immobilized antibody may be determined by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

Nucleic acid assays may also be used to detect for the presence of DNA and RNA for a relaxin-3 chimeric polypeptide polynucleotide, or GPCR135 or GPCR142, in a sample. Exemplary embodiments include suitable techniques known in the art such as Southern analysis, Northern analysis, dot blots, RNase protection, S 1 analysis, amplification techniques such as PCR and LCR, RTPCR, and *in situ* hybridization. In *in situ*

hybridization, the target nucleic acid is liberated from
its cellular surroundings in such a way as to be
available for hybridization within the cell while
preserving the cellular morphology for subsequent
interpretation and analysis. See also, Singer et al.,
1986, *Biotechniques*, 4:230-250; Haase et al., 1984,
Methods in Virology, 7:189-226; and NUCLEIC ACID
HYBRIDIZATION: A PRACTICAL APPROACH (Hames et al., eds. 1987).

    As mentioned above, the chimeric ligand component in
the present invention can also be produced by known
procedures for peptide synthesis. The methods for
peptide synthesis may be selected from suitable solid-
phase synthesis and liquid-phase synthesis techniques.
For example, the desired peptide can be produced by
condensing a partial peptide or amino acid capable of
constituting the protein with the residual part thereof
and, when the product has a protective group, removing
the protective group. Methods for condensation and
deprotection during peptide synthesis are described in
literature, for example, in: Bodanszky and Ondetti,
"Peptide Synthesis," Interscience Publishers, New York,
1966; Schroeder and Luebke, "The Peptide," Academic
Press, New York, 1965; Izumiya et al., "Fundamentals and
Experiments in Peptide Synthesis," Maruzen, 1975; Yajima
and Sakakibara, "Biochemical Experiment Series 1, Protein
Chemistry IV," 205, 1977; and Yajima (ed.), "Development
of Drugs-Continued, 14, Peptide Synthesis," Hirokawa
Shoten.
    After the peptide synthesis reaction, the protein
product can be purified and isolated by a suitable
combination of conventional purification techniques, such
as solvent extraction, column chromatography, liquid
chromatography, and recrystallization. Where the protein

isolated is in a free form, it can be converted to a
suitable salt by a known method. Conversely, where the
isolated product is a salt, it can be converted to the
free peptide by a suitable method selected from those
known in the art.

The amide of a polypeptide can be obtained by using
a resin for peptide synthesis that is suited for
amidation. Exemplary resins include chloromethyl resin,
hydroxymethyl resin, benzhydrylamine resin, aminomethyl
resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenz-
hydrylamine resin, PAM resin, 4-
hydroxymethylmethylphenyl-acetamidomethyl resin,
polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-
hydroxymethyl) phenoxy resin, and 4-(2',4'-
dimethoxyphenyl-Fmoc aminoethyl)phenoxy resin. Using
such a resin, amino acids whose □-amino groups and
functional side-chain groups (R groups) have been
suitably protected are condensed on the resin according
to the sequence of the objective peptide by various
condensation techniques that are known to those skilled
in the art. At the end of the series of reactions, the
peptide or the protected peptide is separated from the
resin and the protective groups are removed to obtain the
objective polypeptide.

The sample comprising the GPRC135 receptor, the
GPCR142 receptor, or both receptors can comprise intact
host cells with the receptor(s) expressed on the cell-
surface, isolated cell membranes from host cells of the
receptor(s), or a purified fragment of the receptor(s)
that is capable of binding to the ligand. An endogenous
host cell for the GPCR135 receptor or the GPCR142
receptor can also be used. In certain preferred
embodiments, an endogenous host cell comprising a

GPCR135, a GPCR142, or both a GPCR135 and a GPCR142 is used.

It is known that a GPCR binds to its endogenous ligand sometimes with its extracellular domain. Such a binding domain can be identified by various methods known to those skilled in the art, such as sequence analyses, protein-protein interaction analyses, protein structural analyses, or a combination of these methods. For example, the ligand binding domain in metabotropic glutamate receptors has been identified as a Venus flytrap module (VFTM) in its extracellular domain (O'Hara et al., 1993, *Neuron*, 11(1):41-52; David et al., 1999, *J. Biol. Chem.*, 274:33488-33495). In a preferred embodiment, the ligand-component binding domain of either GPCR135 or GPCR142 can be first identified using the above methods, and such a ligand-component binding domain can be recombinantly expressed, purified and used in forming a complex of the invention.

In certain embodiments, the inventive relaxin-3 chimeric polypeptides are recombinantly produced, e.g., as secreted proteins composed of two chains (A-chain and B-chain) of polypeptides linked by disulfide bridges, where proteases are involved in the production of mature chimeric polypeptides from their immature pre-propolypeptide precursors. Signal sequence peptidase cleaves off the signal sequence from the pre-propolypeptide, resulting in a relaxin-3 chimeric propolypeptide. The relaxin-3 chimeric propolypeptide is subsequently cleaved into chains A, B, and C by pro-hormone convertases. The so-cleaved A-chain and B-chain for a given chimeric polypeptide are bonded by the formation of disulfide linkages to form the mature relaxin-3 chimeric polypeptide; the C-chain is removed and is not a portion of the mature relaxin-3 chimeric

polypeptide. Because pro-hormone convertases are only selectively expressed in certain cell types where hormones are secreted, such as some neuron cells or endocrine cells, the relaxin-3 chimeric propeptide is often not processed to an optimal efficiency when it is recombinantly expressed from another cell type, such as a COS7.

A "linkage" or "linked" refers to a bond or connective force by which two or more chemical entities, such as polypeptides or polynucleotides, are joined or otherwise associated, such as via the formation of one or a plurality of covalent bonds, ionic bonds, electrostatic bonds, hydrogen bonds, London dispersion forces, Van der Waals interactions, or hydrophobic interactions. Preferably, the linkages employed in preparing the inventive relaxin-3 chimeric polypeptides are effected by the formation of covalent bonds.

"Prohormone convertase (PC)" refers to a family of Ca^{2+}-dependent serine proteases, all of which possess homology to the bacterial endoproteases subtilisin (bacteria) and yeast kexin. This family, also known as furin/paired basic amino-acid-cleaving enzyme (PACE), includes, e.g., PC1/PC3, PC2, PC4, PACE4, PC5/PC6, and PC7/PC8/lymphoma proprotein convertase, and SKI-1. They share a degree of amino-acid identity of 50-75% within their catalytic domains (for a review on PCs, see Seidah et al., 1999, *Brain Res*, 848(1-2): 45-62).

The relaxin-3 chimeric polypeptide may be produced from a recombinant host cell by steps comprising: constructing a DNA molecule capable of encoding a relaxin-3 chimeric pre-propolypeptide, comprising a polynucleotide encoding a relaxin-3 B-chain joined by a first peptide linkage to a relaxin-3 C-chain, the C-chain further joined by a second peptide linkage to a

relaxin/insulin family member A-chain, and a protease
cleavage site inserted at at least one location selected
from the group consisting of the second peptide linkage
between the A-chain and C-chain and the first peptide
linkage between the C-chain and the B-chain of the
relaxin-3 pre-propolypeptide; constructing a vector
capable of expressing the relaxin-3 pre-propolypeptide;
constructing another vector capable of expressing a
protease that can cleave the relaxin-3 pre-propolypeptide
at the inserted protease cleavage site(s); introducing
both the vectors into a host cell; and growing the host
cell under conditions suitable for the expression of both
the modified relaxin-3 pre-propolypeptide and the
protease, so that the protease will cleave a peptide
linkage at the inserted protease cleavage site(s) on the
relaxin-3 chimeric pre-propolypeptide yielding a mature
relaxin-3 chimeric polypeptide.

Sequence analyses suggested that furin, a member of
the pro-hormone convertases, cleaves the peptide linkage
between chains C and B on the native relaxin-3 pre-
propolypeptide, and another unidentified protease cleaves
the peptide linkage between chains A and C on the native
propeptide. A furin cleavage site, with the amino acid
sequence of arg-gly-arg-arg (RGRR), may be inserted at
the peptide junction of chains A and C. An expression
vector for this mutant relaxin-3 chimeric
prepropolypeptide and another expression vector for furin
are co-transfected into a host cell. Under suitable
growth conditions, the relaxin-3 chimeric pre-
propolypeptide is almost completely processed into mature
polypeptides, A, B, and C. Instead of furin, other
proteases can also be used. Identical protease cleavage
sites can be inserted between chains A and C, and chains
C and B, of the chimeric pre-propolypeptide. Examples of

proteases that can be used include, e.g., a pro-hormone convertase, such as furin, PC1, and PC2 (Hosaka et. al., 1991, *J. Biol. Chem.*, 266:12127-12130; Benjannet et. al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:3564-3568; Thomas et. al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:5297-5301).

In preferred embodiments, a tag, such as a HA, poly His, or FLAG, can be added either to the inventive modified relaxin-3 chimeric pre-propolypeptides. The term "tag" refers to an amino acid sequence or a nucleotide sequence encoding an amino acid sequence that facilitates isolation, purification or detection of a protein containing a tag. A variety of such tags are known to those skilled in the art and are suitable for use in the present invention. Suitable tags include, e.g., HA peptide, polyhistidine peptides, biotin/avidin, FLAG, and a variety of antibody epitope binding sites. Techniques known to modify a DNA molecule to cause certain desirable changes in the amino acid sequence encoded by such DNA molecule, such as PCR, may be employed. In a particularly preferred embodiment, a FLAG tag is added to the inventive relaxin-3 chimeric polypeptides.

The identified interaction between the inventive relaxin-3 chimeric polypeptides and GPCR135 and GPCR142 allows for the employment of either GPCR135 or GPCR142, or GPCR135/relaxin-3 chimeric polypeptide complexes or GPCR142/relaxin-3 chimeric polypeptide complexes, in screening methods or assays for identifying compounds for their potential efficacy or selectivity in modulating the activity of such receptors or such receptor-ligand complexes.

Thus, another general aspect of the invention relates to a method for a identifying a compound that

modulates a biological activity of at least one receptor
selected from the group consisting of GPCR135 and
GPCR142, comprising the steps of: (a) contacting a test
sample comprising a compound with an assay reagent
comprising the at least one receptor and a relaxin-3
chimeric polypeptide as defined in; (b) determining the
biological activity of the at least one receptor after
performing step (a); and (c) comparing the biological
activity determined in step (b) with a control
measurement obtained by contacting a control sample not
containing the compound with the assay reagent.

In a preferred embodiment of the invention, the
determination of biological activity comprises
identifying the formation of a receptor-ligand complex.
The receptor ligand complexes of the invention can be
formed by adding the ligand component to a receptor
component-expressing host cell in the form of a purified
protein, or in the form of a cell or tissue extract
containing the ligand component, as described above.

A variety of labels can be used to label the ligand
component, such as those that provide direct detection
(e.g., radioactivity, luminescence, optical or electron
density), or indirect detection (e.g., epitope tag such
as the FLAG epitope, or enzyme tag such as horseradish
peroxidase). In a particularly preferred embodiment, the
ligand component is radiolabeled and the identification
of the formation of a receptor-ligand complex is
performed by radiography.

In preferred embodiments of the invention, the
GPCR135 or GPCR142, or GPCR135 and GPCR142, is a
component of a membrane on the cell surface of a host
cell.

In other preferred embodiments, the modulation of a biological activity of the at least one receptor described above is determined by measuring a second messenger response of a host cell expressing at least of receptor selected from the group consisting of GPCR135 and GPCR142, or isolated membranes comprising such at least one receptor. For example, the biological activity of the complex can be measured by the signal transduction event triggered by activated GPCR135 or GPCR142. This signal transduction event can be measured indirectly by means of measuring one or more changes in cellular physiology, such as cell morphology, migration, or chemotaxis, using one or more suitable methods known in the art. It can also be measured directly by measuring phosphorylation of proteins involved in the signal transduction pathway, for example, the phosphorylation of a GTP-binding protein (G protein). Methods are known in the art for measuring protein phosphorylation, for example, by using an ATP or GTP molecule that has been radiolabeled on the γ-phosphate. A variety of labels can be used to label the GTP molecule on the γ-phosphate, such as a fluorescent molecule or a radioactive isotope such as ^{35}S, ^{32}P, and the like.

The biological activity of the inventive receptor/ligand complexes can also be measured by the intracellular concentration of a second messenger molecule using any of a number of suitable techniques known in the art. For example, the pH change can be measured using a pH-sensitive dye, such as Acridine Orange. The calcium concentration can be measured via optical imaging of fluorescent indicators sensitive to Ca^{2+}, such as fluo-3 (pentapotassium salt, cell-impermeant form; Molecular Probes) or fluo-3(AM) (an acetoxymethyl ester form of fluo-3, Teflabs) (see for example, Liu et

al., 2001, *J. Pharmacol. Exp. Ther.*, 299:121-130) using a
fluorometric imaging plate reader (FLIPR) or a confocal
microscope. The cAMP concentration can be detected using
a commercially available ELISA kit (FLASHPLATE cyclic AMP
assay system (^{125}I), Cat. No: SMP001A, NEN; see also
Shimomura et al., 2002, *J. Biol. Chem.*, 277: 35826-
35832), or via a reporter system wherein the expression
of a reporter gene, such as beta-galactosidase, is under
the control of a cAMP responsive element (cre) (Montminy
et al., 1990, *Trends Neurosci.*, 13(5):184-188).

The test compound can be further characterized by
comparing its effect on two cells, the first cell
containing a functional GPCR135 or GPCR142, or both a
GPCR135 and a GPCR142, and the second one identical to
the first, but lacking a functional GPCR135 or GPCR142,
or lacking both GPCR135 and GPCR142. This technique is
also useful in establishing the background noise of these
assays. Skilled artisans in the art will appreciate that
this control mechanism also allows ready selection of
cellular changes that are responsive to modulation of
functional GPCR135 or GPCR142, or GPCR135 and GPCR142.
Therefore, in preferred exemplary embodiments, the
screening method comprises the steps of: (a) contacting a
first cell having a GPCR135 or a GPCR142, or both a
GPCR135 and a GPCR142, expressed on the cell surface with
a ligand component and with a test compound; (b)
determining a second messenger response in the first cell
to the test compound, and comparing it with that of a
control wherein the first cell is only contacted with the
ligand component, but not the test compound; (c)
contacting a second cell with the ligand component and
with a test compound; wherein the second cell is
otherwise identical to the first cell except that it does
not express one or both of the GPCR135 or the GPCR142 on

the cell surface; (d) determining a second messenger response of the second cell to the test compound, and comparing the second messenger response with that of a control wherein the second cell is only contacted with the ligand component, but not the test compound; and (e) comparing the comparison result of (b) with that of (d).

There are a number of ways to obtain two cells that are otherwise identical except that one expresses a receptor component on its cell surface and the other does not. In one embodiment, the first cell is a recombinant host cell for either GPCR135 or GPCR142 that constitutively expresses either GPCR135 or GPCR142 on its cell surface, and the second cell is the parent cell from which the GPCR135 or GPCR142 recombinant cell is constructed. In another embodiment, a recombinant host cell for either GPCR135 or GPCR142 is constructed such that its expression on the cell surface is under the control of an inducible promoter. The first cell is the recombinant cell grown under inducible conditions that allows the expression of either GPCR135 or GPCR142 on its cell surface, and the second cell is the recombinant cell grown under non-inducible conditions that do not allow the expression of either GPCR135 or GPCR142. In yet another embodiment, the first cell is a native host cell for either GPCR135 or GPCR142 that expresses the polypeptide on its cell surface, and the second cell is a mutant cell derived from the native host, wherein either the GPCR135 or the GPCR142 gene has been inactivated through mutagenesis. Standard molecular biology methods can be used to construct a recombinant host cell for either GPCR135 or GPCR142, or to inactivate either a GPCR135 gene or a GPCR142 gene.

In other preferred embodiments of the invention, the assay methods described above are employed with an

isolated membrane of which the GPCR135 or GPCR142, or

GPCR135 and GPCR142, is a component. The membrane can be

isolated from a native host cell that expresses either

GPCR135 or GPCR142, or both GPCR135 and GPCR142, on its

cell surface, or preferably, from a recombinant host cell

that expresses an increased amount such receptor(s) on

its cell surface. It can also be isolated from tissues

comprising GPCR135 or GPCR142 host cells. Alternatively,

synthetic membranes comprising the receptor(s) can be

prepared by means that are available in the art.

Active compounds or modulators, identified as

described above, as well as the inventive polypeptides,

polynucleotides, and expression vectors, may be used as

therapeutic agents in treating a subject suffering from a

disease or disorder mediated through modulation of

GPCR135 and/or GPCR142, or the interaction of relaxin-3

or ligand with such receptor(s), or the receptor/ligand

complex(es). Such diseases or disorders may include,

e.g., CNS disorders (anxiety, schizophrenia, depression,

mood, sleep/wake), metabolic disorders, feeding/drinking

disorders, water and nutrient homeostasis, and endocrine

disorders, aural sensory processing disorders, visual

attention and saccadic eye movement disorders, disorders

associated with processing of tactile stimuli, disorders

associated with sensory processing, integration, and

motor control, and disorders associated with regulatory

sensory perception, sensory-motor reflexes under

stressful conditions, and disorders associated with

aberrant functional activity of the lateral septum,

hebenula, superior colliculus, inferior colliculus,

amygdala, thalamus, interpeduncular nucleus,

hypothalamus, supraoptic nucleus, dorsal raphe, dentate

gyrus, olfactory bulb, and solitary tract. Such

modulators may also be used to treat reproductive disorders and dysfunctions, such as infertility, and may be employed as contraceptive agents. See e.g., Goto et al., 2001, *J. Comp. Neurol.*, 438:86-122; Osheroff et al., 1991, *Proceedings of the National Academy of the Sciences USA*, 88(15):6413-6417; Adams et al., 2000, *Brain Research*, 858(1):177-180; Liu et al., 2003, *Journal of Biological Chemistry* 278(50):50754-50764; Chalmers et al., 1995, *Journal of Neuroscience* 15(10):6340-6350; Potter et al., 1994, *Proceedings of the National Academy of the Sciences USA* 91:8777-8781; Van Pett et al., 2000, *Journal of Comparative Neurology*, 428:191-212).

Modulators that decrease a biological activity are often referred to as inhibitors, which include compounds that decrease, prevent, inactivate, desensitize, antagonize, repress, corepress, abrogate, down-regulate or compete with receptor or ligand expression, activity or interaction (e.g., GPCR135/relaxin-3 chimeric polypeptide complex expression or activity GPCR142/relaxin-3 chimeric polypeptide complex expression or activity, or expression or activity of both receptor/relaxin-3 chimeric polypeptide complexes or a receptor or ligand individually). Modulators that increase a biological activity are often referred to as activators, which include compounds that increase, effect, activate, coactivate, sensitize, agonize, stimulate, facilitate, promote, sensitize or up-regulate receptor and/or ligand expression, activity or interaction. "Modulators" are intended to include both "inhibitors" and "activators," as well as agonists, antagonists, and inverse agonists.

The compound identification methods can be performed using conventional laboratory formats or in assays

adapted for high throughput. The term "high throughput" refers to an assay design that allows easy screening of multiple samples simultaneously or single samples rapidly, and can include the capacity for robotic manipulation. Another desired feature of high throughput assays is an assay design that is optimized to reduce reagent usage, or minimize the number of manipulations in order to achieve the analysis desired. Examples of assay formats include 96-well or 384-well plates, levitating droplets, and "lab on a chip" microchannel chips used for liquid-handling experiments. Of course, as miniaturization of plastic molds and liquid-handling devices are advanced, or as improved assay devices are designed, greater numbers of samples will be able to be screened more efficiently using the inventive assay.

Candidate compounds for screening can be selected from numerous chemical classes, preferably from classes of organic compounds. Although candidate compounds can be macromolecules, preferably the candidate compounds are small-molecule organic compounds, i.e., those having a molecular weight of greater than 50 and less than 2500. Candidate compounds have one or more functional chemical groups necessary for structural interactions with polypeptides. Preferred candidate compounds have at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two such functional groups, and more preferably at least three such functional groups. The candidate compounds can comprise cyclic carbon or heterocyclic structural moieties and/or aromatic or polyaromatic structural moieties substituted with one or more of the above-exemplified functional groups. Candidate compounds also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs

of the above, or combinations thereof and the like. Where the compound is a nucleic acid, the compound is preferably a DNA or RNA molecule, although modified nucleic acids having non-natural bonds or subunits are also contemplated.

Candidate compounds may be obtained from a variety of sources, including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Candidate compounds can also be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid-phase or solution-phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection (see, e.g., Lam, 1997, *Anti-Cancer Drug Des.*, 12:145). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or may be routinely produced. Additionally, natural and synthetically produced libraries and compounds can be routinely modified through conventional chemical, physical, and biochemical means.

Furthermore, known pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, and amidification to produce structural analogs of the agents. Candidate compounds can be selected randomly or can be based on existing compounds that bind to and/or modulate the function or activity of a GPCR or of one of

the inventive relaxin-3 chimeric polypeptides.
Therefore, a source of candidate agents is known or
screened libraries of molecules including activators or
inhibitors of GPCRs with similar structures to GPCR135,
GPCR142, or the inventive relaxin-3 chimeric
polypeptides. The structures of such compounds may be
changed at one or more positions of the molecule to
contain more or fewer chemical moieties or different
chemical moieties. The structural changes made to the
molecules in creating the libraries of analog
activators/inhibitors can be directed, random, or a
combination of both directed and random substitutions
and/or additions.

A variety of other reagents also can be included in
the assay mixture. These include reagents such as salts,
buffers, neutral proteins (e.g., albumin), and detergents
that can be used to facilitate optimal protein-protein
and/or protein-nucleic acid binding. Such a reagent can
also reduce non-specific or background interactions of
the reaction components. Other reagents that improve the
efficiency of the assay, such as nuclease inhibitors,
antimicrobial agents, and the like, can also be used.

Examples of methods for the synthesis of molecular
libraries can be found in the art, for example in
Zuckermann et al., 1994, *J. Med. Chem.*, 37:2678.
Libraries of compounds can be presented in solution
(e.g., Houghten, 1992, *Biotechniques*, 13:412-421), or on
beads (Lam, 1991, *Nature*, 354:82-84), chips (Fodor, 1993,
Nature, 364:555-556), bacteria (U.S. Patent No.
5,223,409), spores (U.S. Patent No. 5,571,698), plasmids
(Cull et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:1865-
1869) or phage (see e.g., Scott et al., 1990, *Science*,
249:386-390).

Thus, in one general aspect the invention relates to a method of identifying a compound that binds to at least one receptor selected from the group consisting of GPCR135 and GPCR142, comprising the steps of: (a) contacting the at least one receptor with a test compound and with a labeled relaxin-3 chimeric polypeptide; (b) determining the amount of the labeled relaxin-3 chimeric polypeptide that binds to the at least one receptor; and (c) comparing the amount determined in step (b) with a control measurement obtained by contacting the at least one receptor with the labeled relaxin-3 chimeric polypeptide in the absence of the test compound.

In yet other embodiments, the present invention provides a method of identifying a compound that binds to at least one receptor selected from the group consisting of GPCR135 and GPCR142, comprising the steps of: a) contacting a receptor component with a test compound and with a labeled ligand component; b) measuring the amount of the labeled ligand component that binds to the at least on receptor; and c) comparing the measured amount of (b) with that of a control measurement obtained by contacting the receptor(s) with the labeled ligand component in the absence of the test compound.

In one preferred exemplary embodiment, a receptor host cell (recombinant or native) that expresses the at least one receptor on the cell surface can be used for the binding assay. In another preferred exemplary embodiment, isolated membrane preparations comprising the at least one receptor can be used for the binding assay. In yet another preferred embodiment, a substantially purified extracellular fragment of the at least one receptor that is capable of binding to an inventive relaxin-3 chimeric polypeptide can be used for the binding assay.

The amount of the labeled ligand component or
fragment thereof that binds to the at least one receptor
can be measured by first separating the unbound labeled
ligand component or fragment the at least one receptor,
and then measuring the amount of labeling that is
associated with the at least one receptor.

Separation of the at least one receptor protein from
unbound labeled ligand components can be accomplished in
a variety of ways. Conveniently, the at least one
receptor may be immobilized on a solid substrate, from
which the ligand component can be easily separated. The
solid substrate can be made of a variety of materials and
in a variety of shapes, e.g., microtiter plate,
microbead, dipstick, and resin particle. The substrate
preferably is chosen to maximize signal-to-noise ratios,
primarily to minimize background binding, as well as for
ease of separation and cost.

Separation can be effected by, for example, removing
a bead or dipstick from a reservoir, emptying or diluting
a reservoir such as a microtiter plate well, or rinsing a
bead, particle, chromatographic column or filter with a
wash solution or solvent. The separation step preferably
includes multiple rinses or washes. For example, when the
solid substrate is a microtiter plate, the wells can be
washed several times with a washing solution, e.g., that
includes those components of the incubation mixture that
do not participate in specific bindings, such as salts,
buffer, detergent, non-specific protein, etc. Where the
solid substrate is a magnetic bead, the beads can be
washed one or more times with a washing solution and
isolated using a magnet.

The at least one receptor can be immobilized on a
solid substrate using a number of methods. In one
embodiment, a fusion protein can be provided which adds a

domain that allows the at least one receptor proteins to be bound to a matrix. For example, glutathione-S-transferase fusion proteins or glutathione-S- transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound and the labeled ligand component, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding of the labeled ligand component to the at least one receptor can be determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, the at least one receptor can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated polypeptide can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit available from Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96-well plates (Pierce Chemicals).

Alternatively, antibodies reactive with the receptor(s) but which do not interfere with binding of the at least one receptor to the ligand component or test compound can be attached to the wells of the plate, and the at least one receptor then trapped in the wells by antibody conjugation.

A variety of labels can be used to label either the ligand component or fragments thereof, such as those that provide direct detection (e.g., radioactivity, luminescence, optical or electron density), or indirect detection (e.g., epitope tag such as the FLAG epitope, or enzyme tag such as horseradish peroxidase).

Interaction of the at least one receptor to the ligand component in the presence and absence of a candidate compound can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes.

Further, the receptor can be modified to produce constitutive activity. This constitutive activity can be suppressed by an inverse agonist.

In certain embodiments or the present invention, methods are provided for treating a medical condition mediated by modulation of receptor or ligand activity, expression or interaction, comprising administering to a subject in need thereof a therapeutically effective amount of a compound identified as having such modulating activity. The term "treating" includes preventing, decreasing, diminishing, suppressing, alleviating, or ameliorating the symptoms or causes of a medical condition. Such treatment can be therapeutic or prophylactic. The term "subject" as used herein refers to an animal who is the object of treatment, observation or experiment. Preferably, the subject is a mammal, more preferably a human.

A "pharmaceutically effective amount" is an amount or dose sufficient to achieve the desired modulating effect. Suitable amounts for a particular treatment may be ascertained by routine methods such as modeling, dose escalation studies or clinical trials, and by taking into

consideration routine factors, e.g., the mode or route of administration or drug delivery, the pharmacokinetics of the agent, the severity and course of the disorder or condition, the subject's previous or ongoing therapy, the subject's health status and response to drugs, and the judgment of the treating physician. An exemplary dose is in the range of from about 0.001 to about 200 mg per kg of subject's body weight per day, preferably about 0.05 to 100 mg/kg/day, or about 1 to 35 mg/kg/day, in single or divided dosage units (e.g., BID, TID, QID). For a 70-kg human, an illustrative dosage amount is from about 0.05 to about 7 g/day, or about 0.2 to about 2.5 g/day.

Once improvement of the patient's conditions has occurred, the dose may be adjusted for preventative or maintenance treatment. For example, the dosage or the frequency of administration, or both, may be reduced as a function of the symptoms, to a level at which the desired therapeutic or prophylactic effect is maintained. Of course, if symptoms have been alleviated to an appropriate level, treatment may cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of symptoms.

The compounds or treatment agents of the invention may be used alone or in combination with one or more other active ingredients, to formulate pharmaceutical compositions of the invention. A pharmaceutical composition of the invention comprises a pharmaceutically effective amount of a compound having modulating activity, which can be determined using methods as described above. A "pharmaceutically acceptable excipient" refers to a substance that is not toxic, biologically intolerable, or otherwise biologically unsuitable for administration to a subject, such as an inert substance, added to a pharmacological composition

or otherwise used as a vehicle, carrier, or diluent to facilitate administration of a pharmaceutical agent and that is compatible therewith. Examples of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Delivery forms of the pharmaceutical compositions containing one or more dosage units of the pharmaceutical agents may be prepared using suitable pharmaceutical excipients and compounding techniques known to those skilled in the art. The compositions may be administered in the inventive methods by oral, parenteral, rectal, topical, or ocular routes or by inhalation.

The preparation may be in the form of tablets, capsules, sachets, dragees, powders, granules, lozenges, powders for reconstitution, liquid preparations, or suppositories. Preferably, the compositions are formulated for intravenous infusion, topical administration, or oral administration.

For oral administration, the compounds of the invention can be provided in the form of tablets or capsules, or as a solution, emulsion, or suspension. To prepare the oral compositions, the agents may be formulated to yield a dosage of, e.g., from about 0.05 to about 50 mg/kg daily, or from about 0.05 to about 20 mg/kg daily, or from about 0.1 to about 10 mg/kg daily.

Oral tablets may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives agents. Suitable inert fillers include sodium and calcium carbonate, sodium and calcium phosphate, lactose, starch, sugar, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol,

and the like. Exemplary liquid oral excipients include
ethanol, glycerol, water and the like. Starch,
polyvinyl-pyrrolidone (PVP), sodium starch glycolate,
microcrystalline cellulose, and alginic acid are suitable
disintegrating agents. Binding agents may include starch
and gelatin. The lubricating agent, if present, may be
magnesium stearate, stearic acid or talc. If desired,
the tablets may be coated with a material such as
glyceryl monostearate or glyceryl distearate to delay
absorption in the gastrointestinal tract, or may be
coated with an enteric coating.

Capsules for oral administration include hard and
soft gelatin capsules. To prepare hard gelatin capsules,
active ingredient may be mixed with a solid, semi-solid,
or liquid diluent. Soft gelatin capsules may be prepared
by mixing the active ingredient with water, an oil such
as peanut oil or olive oil, liquid paraffin, a mixture of
mono and di-glycerides of short chain fatty acids,
polyethylene glycol 400, or propylene glycol.

Liquids for oral administration may be in the form
of suspensions, solutions, emulsions or syrups or may be
presented as a dry product for reconstitution with water
or other suitable vehicle before use. Such liquid
compositions may optionally contain: pharmaceutically-
acceptable excipients such as suspending agents (for
example, sorbitol, methyl cellulose, sodium alginate,
gelatin, hydroxyethylcellulose, carboxymethylcellulose,
aluminum stearate gel and the like); non-aqueous
vehicles, e.g., oil (for example, almond oil or
fractionated coconut oil), propylene glycol, ethyl
alcohol or water; preservatives (for example, methyl or
propyl p-hydroxybenzoate or sorbic acid); wetting agents
such as lecithin; and, if desired, flavoring or coloring
agents.

The compositions of this invention may also be administered by non-oral routes. For example, the compositions may be formulated for rectal administration as a suppository. For parenteral use, including intravenous, intramuscular, intraperitoneal, or subcutaneous routes, the agents of the invention may be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity or in parenterally acceptable oil. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Such forms will be presented in unit-dose form such as ampules or disposable injection devices, in multi-dose forms such as vials from which the appropriate dose may be withdrawn, or in a solid form or pre-concentrate that can be used to prepare an injectable formulation. Illustrative infusion doses may range from about 1 to 1000 µg/kg/minute of agent, admixed with a pharmaceutical carrier over a period ranging from several minutes to several days.

For topical administration, the agents may be mixed with a pharmaceutical carrier at a concentration of about 0.1% to about 10% of drug to vehicle. Another mode of administering the agents of the invention may utilize a patch formulation to affect transdermal delivery.

Agents may alternatively be administered in methods of this invention by inhalation, via the nasal or oral routes, e.g., in a spray formulation also containing a suitable carrier.

Another embodiment of the invention provides a method for treating a medical condition comprising administering a pharmaceutically acceptable composition comprising a pharmaceutically acceptable vector of the invention. Polynucleotides of the invention may be

introduced into a cell, *in vitro*, *in vivo*, or *ex vivo*, using any suitable method, e.g., infection with viral vectors, liposome-based methods, biolistic particle acceleration, and naked DNA injection.

Such therapeutically useful nucleic acids include coding sequences for biologically active protein. Such a sequence may be operably linked to a promoter as described above. The nucleic acid may be administered in the form of a cell that is itself directly therapeutically effective, e.g., certain antisense or ribozyme molecules. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-*O*-alkylRNA, or other antisense oligonucleotide mimetics. Antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence.

Gene therapy techniques may be used to introduce the biologically active protein into the cells of target organisms. To illustrate, the DNA is ligated into viral vectors that mediate transfer of the DNA by infection of recipient host cells. Suitable exemplary viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, poliovirus and the like. Alternatively, the DNA can be transferred into cells for gene therapy by non-viral techniques, such as receptor-mediated targeted DNA transfer using ligand-DNA conjugates or adenovirus-ligand-DNA conjugates, lipofection membrane fusion, or direct microinjection. Protocols for molecular methodology of gene therapy suitable for use with the gene of interest are

exemplified in GENE THERAPY PROTOCOLS, edited by Paul D.
Robbins, Human press, Totawa NJ, 1996.

Pharmaceutically effective compositions comprising
DNA, RNA, or polypeptides, or small-molecule modulators
of relaxin-3 or receptor activity, may be formulated
according to known methods such as by the admixture of
a pharmaceutically acceptable carrier. Examples of
such carriers and methods of formulation may be found
in REMINGTON'S PHARMACEUTICAL SCIENCES. To form a
pharmaceutically acceptable composition suitable for
effective administration, such compositions will
contain an effective amount of the polypeptide, DNA,
RNA, or modulator.

Other illustrative features and preferred
embodiments of the invention are described in the
following examples.

EXAMPLE 1

Cloning, Expression and Purification of Relaxin-3
Chimeric Polypeptides, GPCRs, LGRs, and Expression
Vectors Encoding Same

**Construction of chimeric polypeptide expression
constructs**

A series of chimeric polypeptides were prepared by
creating gene cassettes which included coding regions for
an alpha peptide signal peptide, a FLAG tag, the human
relaxin-3 B-chain, the human relaxin-3 C-chain, and an A-
chain from one of the following: relaxin-1 (Hudson et
al., 1983, *Nature*, 301:628-631), relaxin-2 (Hudson et
al., 1984, *EMBO Journal*, 3:2333-2339), INSL3 (Adham, et
al., 1993, *Journal of Biological Chemistry* 268:26668-

26672), INSL4 (Koman et al., 1996, *Journal of Biological Chemistry*, 271:20238-20241), INSL5 (Conklin et al., 1999, *Genomics*, 60:50-56), INSL6 (Lok et al., 2000, *Biology of Reproduction*, 62:1593-1599), insulin, or an artificial A-chain, in which only the cysteine residues remained unchanged while all the other amino acids residues were randomly assigned. The junction of the C-chain and the A-chain for each construct contained an artificial furin cleavage site (RGRR) derived from the Relaxin-3 RR expression vector from which the coding regions of inventive chimeric polypeptides were PCR amplified (Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764). This furin cleavage sight allowed for efficient *in vivo* cleavage of the chimeric propeptides when co-expressed with furin (Hosaka et al., 1991, *Journal of Biological Chemistry*, 266:12127-12130; Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764).

PCR amplification of the coding regions for the chimeric peptides

A Relaxin-3/Relaxin-1 (R₃/R₁) chimeric polypeptide comprises a relaxin-3 B-chain and a relaxin-1 A-chain. The DNA coding region for such chimeric polypeptide was PCR amplified using modified human relaxin-3 expression vector relaxin-3 RR (Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764) as template and using primers P1 (NO: 32)(5' ACT AGA CTG CAG GCC GCC ATG CTG ACC GCA GCG T 3') and P2 SEQ ID NO:33)(5' ACT AGA GGA TCC TCA GCA ATA TTT AGC AAG AGA CCT TTT GGT ACA ACC AAT TAG GCA ACA TTT CTC AAA CAG TGC CAC GTA GGG TCG TCG TCG GCC CCG AAG AAC CCC AGG 3').

A Relaxin-3/ Relaxin-2 (R₃/R₂) chimeric polypeptide comprises a relaxin-3 B-chain and a relaxin-2 A-chain. The DNA coding region for such chimeric polypeptide was

PCR amplified using modified human relaxin-3 expression
vector Relaxin-3 RR (see above) as template and using
primers P1 (SEQ ID NO:32 above) described above and P3
(SEQ ID NO:34)(5' ACT AGA GGA TCC TCA GCA AAA TCT AGC AAG
AGA TCT TTT GGT ACA ACC AAC ATG GCA ACA TTT ATT AGC CAA
TGC ACT GTA GAG TTG TCG TCG GCC CCG AAG AAC CCC AGG 3').

A Relaxin-3/INSL3 (R₃/I₃) chimeric polypeptide
comprises a relaxin-3 B-chain and an INSL3 A-chain. The
DNA coding region for such chimeric polypeptide was PCR
amplified using modified human relaxin-3 expression
vector Relaxin-3 RR (see above) as template and using
primers P1 (SEQ ID NO:32 above) and P4 (SEQ ID NO:35) (5'
ACG ATA GGA TCC TCA GCA CAG GGT CAG CAG GTC CTG CTG GGT
GCA GCC GGA CAG GCA GCA GTA GCG GGC GGG GTT TCG TCG GCC
CCG AAG AAC CCC AG 3').

A Relaxin-3/INSL4 (R₃/I₄) chimeric polypeptide
comprises a relaxin-3 B-chain and an INSL4 A-chain. The
DNA coding region for such chimeric polypeptide was PCR
amplified using modified human relaxin-3 expression
vector Relaxin-3 RR as template and using primers P1 (SEQ
ID NO:32 above) and P5 (SEQ ID NO:36) (5' ATG ACA GGA TCC
TCA GCA CAG CTT CAC GGA GGT GCC GTC GTC GCA GAT CAC CTC
GCA GCA GAA GGG GTC GAA TCG TCG GCC CCG AAG AAC CCC AGG
3').

A Relaxin-3/INSL5 (R₃/I₅) chimeric polypeptide
comprises a relaxin-3 B-chain and an INSL5 A-chain. The
DNA coding region for such chimeric polypeptide was PCR
amplified using modified human relaxin-3 expression
vector Relaxin-3 RR as template and using primers P1 (SEQ
ID NO:32 above) and P6 (SEQ ID NO:37) (5' ATA GAA GGA TCC
TTA GCA AAG AGC ACT CAA ATC AGT CAT GGA ACA GCC ATC AGT
GCA ACA CAA AGT TTG TAA ATC TTG TCG TCG GCC CCG AAG AAC
CCC AGG GGT TCC 3').

A Relaxin-3/INSL6 (R_3/I_6) chimeric polypeptide comprises a relaxin-3 B-chain and an INSL6 A-chain. The DNA coding region was PCR amplified using modified human relaxin-3 expression vector Relaxin-3 RR as template and using primers P1 (SEQ ID NO:32 above) and P7 (SEQ ID NO:38) (5' ATG ACA GGA TCC TCA GCA GGC GAT GGA CAG CTC CTC CTT GGT GCA GCC GGT CAG GCA GCA CTT CTC GGA GTA GCC TCG TCG GCC CCG AAG AAC CCC AG 3').

A Relaxin-3/Insulin (R_3/I) chimeric polypeptide comprises a relaxin-3 B-chain and an insulin A-chain. The DNA coding region was PCR amplified using modified human relaxin-3 expression vector Relaxin-3 RR as template and using primers P1 (SEQ ID NO:32 above) and P8 (SEQ ID NO:39) (5' ACG ATA GGA TCC TCA GCA GTA GTT CTC CAG CTG GTA CAG GGA GCA GAT GGA GGT GCA GCA CTG CTC CAC GAT GCC TCG TCG GCC CCG AAG AAC CCC AG 3').

A Relaxin-3/artificial A-chain (R_3/A) chimeric polypeptide comprises a relaxin-3 B-chain and an A-chain comprising the conserved cysteine residues and arbitrarily assigned amino acids at the other positions. The DNA coding region was PCR amplified using modified human relaxin-3 expression vector Relaxin-3 RR as template and using primers P1 (SEQ ID NO:32 above) and P9 (SEQ ID NO: 40) (5' ACG ATA GGA TCC TCA GCA GGC CTT CTC GAT CTC GTA GTC GTC GCA GAT GGA CAG GCA GCA GTT CTC GTA CAG GCC TCG TCG GCC CCG AAG AAC CCC AG 3').

Construction of the expression vectors for relaxin-3 chimeric peptides

The PCR products for the relaxin-3 chimeric polypeptide coding regions described supra were digested with DNA restriction enzymes Pst 1 and Bam H1 and cloned into a mammalian expression vector, pCMV sport1 (Invitrogen), between Pst1 and Bam H1 sites. The insert

region for each construct was sequenced to confirm the sequence identity.

Expression and purification of chimeric peptides

The amino acid sequences of the inventive chimeric polypeptides are depicted in Fig. 2.

Each of the different relaxin-3 chimeric polypeptide expression constructs described were co-transfected with a human Furin expression plasmid (Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764) into COS-7 cells. Three days after transfection, recombinant peptides secreted into the conditioned medium of transfected cells were affinity purified with anti-FLAG affinity column, cleaved with enterokinase (Novagen) to remove the N-terminal FLAG tag (Sigma), and then further purified by reversed phase HPLC using a C-18 column. The purified peptides were characterized by SDS-PAGE under non-reducing conditions to verify purity. The protein expression levels for R_3/R_2, R_3/I_3, R_3/I_5, and R_3/I_6 were comparable to the production of relaxin-3 wild type peptides (Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764), which was about 1 mg/L. The production levels of R_3/R_1, R_3/I_4, were lower at proximately 200 µg/L. Attempts to make R_3/I and R_3/A chimeras resulted in no detectable peptides when analyzed by SDS-PAGE.

Molecular Cloning of GPCR135, GPCR142, LGR7, and LGR8

The cloning, preparation and methods of use of recombinant GPRC135 and recombinant GPCR142 are described in Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764, Liu et al., 2003, *Journal of Biological Chemistry*, 278:50765-50770, in patent applications, WIPO publications WO00/23111, WO00/24891, WO01/48189,

WO01/62797, WO01/74904, and WO01/85791, U.S. Provisional Application No. 60/580,083, and U.S. Patent Application No. 10/786,478, the disclosures of which are incorporated herein by reference.

Human LGR7 cDNA containing the complete coding region was PCR amplified from a human brain cDNA pool (BD Biosciences, Palo Alto, CA) using the following two primers: forward primer (SEQ ID NO:41): 5' AGA TGA GAA TTC GCC ACC ATG ACA TCT GGT TCT GTC TTC TTC TAC 3'; and reverse primer (SEQ ID NO:42): 5' TAG AGA GCG GCC GCT CAT GAA TAG GAA TTG AGT CTC GTT GA 3'), each designed according the published sequence (Hsu et al., 2000, *Molecular Endocrinology*, 14:1257-1271; Hsu et al., 2002, *Science*, 295:671-674).

Human LGR8 cDNA containing the complete coding region was PCR amplified from a human testis cDNA pool (BD Biosciences) using the following two primers forward primer (SEQ ID NO:43): 5' TAG ACA GAA TTC GCC ACC ATG ATT GTT TTT CTG GTT TTT AAA CAT CTC 3'; reverse primer (SEQ ID NO:44): 5' ATG ATA GCG GCC GCC TAG GAA ACT GGT TTC ATT ATA CTG TC 3'), each designed according to the published sequence (Hsu et al., 2002, *Science*, 295:671-674). The resulting LGR7 and LGR8 cDNAs were each separately cloned into the mammalian expression vector pCIneo (Promega) and the insert regions were sequenced to confirm the sequence identities for LGR7 and LGR8, respectively.

EXAMPLE 2

Characterization of Relaxin-3 B-chain Agonist Activity Towards GPCR135, GPCR142, LGR7, and LGR8

It has been shown that synthetic human relaxin-3 B-chain alone, but not A-chain, is an agonist for both GPCR135 and GPCR142 (Liu et al., 2003, *Journal of*

Biological Chemistry, 278:50754-50764 and Liu et al.,
2003, *Journal of Biological Chemistry,* 278:50765-50770).
Those studies were repeated herein in SK-N-MC/β-gal
cells, which harbor a β-galactosidase gene under the
control of a cAMP responsive element (CRE) (Liu et al.,
2001, *Molecular Pharmacology,* 59:420-426). In such SK-N-
MC/β-gal cells, an increase in intracellular cAMP
concentration leads to increased β-galactosidase gene
expression, the activity of which is measured using
Chlorophenol Red-β-D-Galactopyranoside (CPRG) as the
substrate.

The results indicate that relaxin-3 B-chain
inhibited forskolin-stimulated β-galactosidase activity
in SK-N-MC/β-gal cells expressing GPCR135 (with an EC_{50}
value of 88 nM, Fig.1A) or GPCR142 (with an EC_{50} value of
125 nM, Fig. 1B) in a dose-dependent manner. In parallel
experiments, human relaxin-3 B-chain did not activate
either LGR7 (Fig. 1C) or LGR8 (Fig.1D) expressing cells,
whereas porcine relaxin and human relaxin-3 stimulated β-
galactosidase activity in LGR7 expressing cells (with EC_{50}
values of 0.25 nM and 1.4 nM, respectively, Fig.1C), and
porcine relaxin and human INSL3 induced β-galactosidase
activity in LGR8 expressing cells (with EC_{50} values of 1.5
nM and 0.16 nM, respectively, Fig. 1D).

EXAMPLE 3

*Pharmacological Characterization of Relaxin-3 Chimeric
Polypeptides by Radioligand Binding*

The inventive chimeric polypeptides were tested as
ligands for GPCR135 (Fig. 3A), GPCR142 (Fig. 3B), LGR7
(Fig. 3C) and LGR8 (Fig. 3D) in radioligand binding
assays to evaluate their receptor binding properties.

Radioligand binding assays for GPCR135 and GPCR142 were performed as previously described (Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764 and Liu et al., 2003, *Journal of Biological Chemistry*, 278:50765-50770). Membranes from COS-7 cells transiently transfected with either the GPCR135 expression vector or the GPCR142 expression vector, each described supra, were incubated with [^{125}I]-relaxin-3 at a final concentration 100 pM in 96-well plates. Peptides for competition binding studies were added to the binding mix (final volume: 200 μl) in binding buffer [50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1% bovine serum albumin, 0.1% protease inhibitor cocktails (Sigma)]. The binding mixtures were incubated at room temperature for 1 hour, filtered through GFC plates (Packard) pre-saturated with 0.3% Polyethylenimine (Sigma), and washed with cold washing buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA). Microscint-40 was added to each well and the bound [^{125}I]-relaxin-3 was counted in a scintillation counter (TopCount/NTX, Packard).

Radioligand binding assays for LGR7 and LGR8 were performed using live COS-7 cells transiently transfected with LGR7 or LGR8 expression plasmids in 15-cm culture dishes using LipofectAmine (Invitrogen). Two days after transfection, the transfected cells were detached from the 15-cm culture dished using phosphate buffered saline (PBS) plus 10 mM EDTA, seeded into 96-well opaque poly-D-lysine coated culture plates and used for radioligand binding assays. For LGR7, [^{125}I]-relaxin-3 was used as the tracer. For LGR8, [^{125}I]-INSL3 (specific activity: 2200 Ci/mmol) was used as the tracer. [^{125}I]-labeled tracer was added in binding reactions at a final concentration of 100 pM in binding buffer containing DMEM plus 50 mM HEPES and 1% bovine serum albumin. Unlabeled relaxin-3 and

relaxin-3 chimeric polypeptides were added at various concentrations to the binding assays as the competitors, as indicated (Fig. 3). The binding mixtures were incubated at room temperature for 1 hour. The unbound radioligand was removed by aspiration of the binding buffer. The cells were washed with PBS. The bound radioligand was counted in a scintillation counter (TopCount/NTX). The results were analyzed using Prism 3.0 program (Graphpad, San Diego, CA). The IC_{50} values represent the ligand concentrations that inhibit 50% of the maximum specific binding.

The IC_{50} values of different chimeric polypeptides for GPCR135, GPCR142, LGR7, and LGR8 are listed in Table 1.

The results showed that all chimeric polypeptides, except R_3/I_4, bound both GPCR135 and GPCR142 with high affinity (i.e., IC_{50} values in the low nanomolar range), but with slight differences in potency. R_3/I_4 demonstrated marginal binding at the highest concentration (1 µM). Chimeric polypeptides R_3/R_1, R_3/R_2, and R_3/I_3, bound to LGR7 with IC_{50} values in low nanomolar range, which is similar to that of relaxin-3. R_3/I_6 bound LGR7 with an IC_{50} value of 15 nM. The R_3/I_5 chimeric polypeptide, which demonstrated affinity for GPCR135 and GPCR142 in the subnanomolar and low nanomolar range, respectively, showed lower affinity for LGR7, with an IC_{50} value of about 0.5 µM. R_3/I_4 was inactive for LGR7 even at the highest concentration tested (1 µM). None of the chimeric polypeptides bound LGR8 with high affinity. R_3/I_4, R_3/I_5, R_3/I_6 showed no binding affinity for LGR8, whereas, chimeric polypeptides R_3/R_1, R_3/R_2, and R_3/I_3, each demonstrated modest binding affinity for LGR8 (IC_{50} values of 0.5 µM to greater than 1 µM).

EXAMPLE 4

Pharmacological Characterization of Relaxin-3 Chimeric Polypeptides in Functional Assays

The inventive chimeric polypeptides were each independently tested for their ability to activate either GPCR135, GPCR142, LGR7, or LGR8, respectively, in separate pools of SK-N-MC/β-gal cells (see supra), each pool stably expressing one of the respective receptors, as indicated (Fig. 4).

Cells were seeded in 96-well tissue culture plate at a density of 30,000 cells/well in MEM plus 10% fetal serum, sodium pyruvate, penicillin, streptomycin, and G418 (400 mg/L). For cells expressing GPCR135 or GPCR142, forskolin was added to cells at a final concentration of 5 μM to stimulate intracellular cAMP accumulation. Different concentrations of each of the inventive polypeptides were then added to the cells to inhibit the forskolin induced cAMP accumulation. For cells expressing LGR7 or LGR8, intracellular cAMP was stimulated with each of the inventive chimeric polypeptides at various concentrations.

For all cells, after adding the chimeric polypeptides, cells were incubated for 6 additional hours in 37°C incubator. The media was then aspirated and the β-galactosidase activities measured as previously described (Liu et al., 2001, *Molecular Pharmacology*, 59:420-426). The results were analyzed by Prism 3.0 software. The EC_{50} values represent the chimeric polypeptide concentrations that achieved 50% of the maximum inhibition of forskolin induced β-galactosidase activity (GPCR135 or GPCR142) or

50% of the maximum chimeric polypeptide induced β-galactosidase activity (LGR7 or LGR8), respectively.

The results demonstrated that all chimeric polypeptides tested act as agonists for both GPCR135 and GPCR142. The chimeric polypeptides, R_3/R_1, R_3/R_2, R_3/I_3, and R_3/I_5, showed similar potency towards GPCR135 and GPCR142 compared to that of the wild type relaxin-3, with EC_{50} values in the low nanomolar range. The chimeric polypeptide, R_3/I_6, was slightly less potent towards both GPCR135 and GPCR142, with EC_{50} values around 10 nM for each receptor. The chimeric polypeptide, R_3/I_4, stimulated GPCR135 or GPCR142 at the highest concentration (1 μM) tested only (Table 2, and Figs. 4A and 4B).

For cells stably expressing LGR7, the results indicated that the chimeric polypeptides, R_3/R_1, R_3/R_2, and R_3/I_3, exhibited high potency with EC_{50} values around 1 to 5 nM. The chimeric polypeptide, R_3/I_6, showed lower potency for LGR7 with an EC_{50} value of 10 nM. The chimeric polypeptide, R_3/I_5, a potent agonist for GPCR135 and GPCR142 (see supra), demonstrated low potency for LGR7, with an EC_{50} value of approximately 400 nM. For cells stably expressing LGR8, the chimeric polypeptide, R_3/R_1, demonstrated marginal activity at the highest concentration tested (1 μM). The R_3/R_2 and R_3/I_3 chimeric polypeptides each showed higher potency with EC_{50} values of about 600 nM and 300 nM, respectively. None of the other chimeric polypeptides tested showed activity towards LGR8 in this assay (Table 2, and Fig.s 4C and 4D).

Control SK-N-MC/β-gal cells not expressing recombinant GPCR135, GPCR142, LGR7, or LGR8 did not respond to any polypeptides.

EXAMPLE 5

Binding of R₃/I₅ to GPCR135-Expressing Cells

Binding of R_3/I_5 to GPCR135-Expressing Cells

All the experiments described in this example were
carried out in accordance with the Guide for the Care and
Use of Laboratory Animals as adopted and promulgated by
the US National Institute of Health.

Adult male Sprague-Dawley rats (150 - 200 g) were
asphyxiated by carbon dioxide. Rat brains were
immediately removed from the skull and rapidly frozen in
dry ice (i.e., solid, subliming CO_2). Twenty-micron-thick
sagital sections were cut using a Cryostat-microtome
(Microm HM505E, Mikron, San Diego, CA) and thaw-mounted
on adhesive microscope slides (Superfrost$^+$ Plus, VWR).
The sections were kept at -70°C until use. Sections were
thawed and dried under a cold air stream and then pre-
incubated for 15 min. at room temperature in incubation
buffer (20 mM HEPES, pH 7.4, 120 mM $NaCl_2$, 0.22 mM KH_2PO_4,
1.3 mM $CaCl_2$, 0.8 mM $MgSO_4$) by immersing sections in a 400
ml jar. Sections were dried again under a cold air
stream and incubated for 60 min. with 5 pM [^{125}I]-relaxin-
3 (specific activity, 2200Ci/mmole) or [^{125}I]-R_3/I_5
(specific activity, 2200 Ci/mmole) in incubation buffer
containing 0.5% BSA and protease inhibitor cocktail
(Sigma). Nonspecific binding was determined in the
presence of 100 nM of human relaxin-3. After incubation,
the excess radioligand was washed off with three
repetitive washes, ten minutes each wash, by immersing
the slides in the jar containing incubation buffer at 4°C
followed by a quick immersion in water. Sections were
dried and exposed to Fujifilm Imaging plates (BAS-MS
2025) for 48 hrs. The imaging plates were scanned using

the Fuji Bio-Imaging Analyzer System (BAS-5000) and
visualized using ImageGauge V3.12 software.

The results showed that while both [^{125}I]-relaxin-3
(Fig. 5A) and [^{125}I]-R$_3$/I$_5$ (Fig. 5B) bind to GPCR135
expressing cells with high affinity, [^{125}I]-R$_3$/I$_5$ produced
lower non-specific binding than [^{125}I]-relaxin-3. In
addition, [^{125}I]-R$_3$/I$_5$ did not demonstrate significant
binding to LGR7, whereas [^{125}I]-relaxin-3 did. When
[^{125}I]-relaxin-3 was used as the radioligand on rat brain
section, no substantial difference between total binding
and non-specific binding (determined in the presence of
100 nM of unlabeled relaxin-3) was observed (Fig 5C). In
contrast, when [^{125}I]-R$_3$/I$_5$ was used as the radioligand,
the non-specific binding was reduced (Fig 5D). GPCR135-
like binding sites were observed in-cortex, olfactory
bulb, and superior colliculus areas (Fig. 5D).

Discussion of results from above examples

GPCR135 is predominantly expressed in the brain
(Matsumoto et al., 2000, *Gene*, 248:183-189; Liu et al.,
2003, *Journal of Biological Chemistry*, 278:50754-50764).
In vivo administration of relaxin-3 in the brain to
activate GPCR135 can be used to study the physiological
role of GPCR135, however, such studies are confounded by
the activation of LGR7 by relaxin-3 (Sudo et al., 2003,
J. Biol. Chem., 278:7855-7862), which is also expressed
in the brain (Tan et al., 1999, *Br. J. Pharmacol.*,
127:91-98; Hsu et al., 2000, *Mol. Endocrinol.*, 14:1257-
1271; Hsu et al., 2002, *Science*, 295:671-674). Selective
ligands that specifically activate GPCR135, but not LGR7,
are desirable to study the *in vivo* function of GPCR135.
Therefore, the inventive relaxin-3 chimeric polypeptides,
e.g., R$_3$/I$_5$, a peptide that displays high affinity for
GPCR135 (K$_d$ ~ 0.5 nM) but low affinity for LGR7 (K$_d$ ~ 0.5

μM), facilitate the *in vivo* functional study of GPCR135, such as in rat, which does not have GPCR142.

In addition to the fact that ^{125}I-relaxin-3 also labels LGR7, the hydrophobic nature of the [^{125}I]-relaxin-3 polypeptide results in a high background when applied in the radioligand autoradiography studies. Addition of the INSL5 A-chain to the B-chain of relaxin-3 resulted in a molecule possessing selectivity for GPCR135 and reduced hydrophobicity, providing lower non-specific binding. Using monolayer cells expressing recombinant GPCR135 or LGR7, [^{125}I]-R$_3$/I$_5$ bound GPCR135 with high affinity and produced similar total binding to that of [^{125}I]-relaxin-3, but with 5 to 10 times less non-specific binding. In addition, [^{125}I]-R$_3$/I$_5$ does not label LGR7 whereas [^{125}I]-relaxin-3 does, confirming [^{125}I]-R$_3$/I$_5$ as an advantageous tool for radioligand autoradiography studies. Direct application of [^{125}I]-R$_3$/I$_5$ in rat brain sections in radioligand autoradiography showed specific binding sites which overlap with the mRNA distribution for GPCR135. Since [^{125}]-R$_3$/I$_5$ applied in trace concentrations does not bind LGR7, [^{125}I]-R$_3$/I$_5$ may be advantageously employed to selectively study GPCR135 receptor autoradiography in the rat brain, which does not express the GPCR142 receptor protein.

The results also showed that at least 200 μg/L of chimeric polypeptides R$_3$/R$_1$, R$_3$/R$_2$, R$_3$/I$_3$, R$_3$/I$_4$, R$_3$/I$_5$, and R$_3$/I$_6$ was produced by the recombinant system employed, indicating that the A-chains from many members of the insulin/relaxin family, although bearing limited sequence conservation except the conserved cysteine residues, are largely inter-exchangeable for protein folding, disulfide bond formation, protein secretion and maintenance of protein stability. However, chimeric peptide R$_3$/A, in which in the A-chain, only the cysteine residues were

left unchanged and all other amino acids were arbitrarily
assigned, was not produced in the recombinant system,
suggesting that certain structural conformation(s) should
be met to yield biologically active relaxin-3 chimera
(e.g., a compatible A-chain should pair with the relaxin-
3 B-chain to allow protein production in the mammalian
cells). The lack of R_3/I chimera production may be
explained by the same reason.

In an effort to understand successful pairing of the
relaxin-3 B-chain with the A-chains from relaxin, INSL3,
INSL4, INSL5, or INSL6 but not the A-chain from insulin,
the amino acid sequence of all known members in the
family were compared (Fig. 6). Amino acid sequence
comparisons for from different members of the
insulin/relaxin family indicated that, except for
insulin, IGF1 and IGF2, the B-chains from other members
of the family have two conserved positively charged amino
acids (Arg or Lys) corresponding to the Arg^8 and Arg^{16} of
the relaxin-3 B-chain. In contrast, the insulin B-chain
has His and Glu in the corresponding positions.
Similarly, both IGF1 and IGF2 have Thr and Asp in the
corresponding positions. These results suggest that
insulin and IGFs may be distinct members of the family
compared to other members. The A-chains from insulin and
IGFs, structurally, may be very distinct from that of
other members, therefore not compatible with relaxin-3 B
chain. Given the evidence that the above-mentioned
positively charged amino acids in relaxin and INSL3 are
important for receptor (namely LGR7 and LGR8) binding and
activation (Bullesbach et al., 1992, *J. Biol. Chem.*,
267:22957-22960; Bullesbach et al., 1999, *Biochemistry*,
38:3073-3078; 2000; Tan et al., 2002, *Eur. J. Pharmacol.*,
457:153-160), it is likely that insulin and IGFs are
distinct members of the family that signal through the

single transmembrane spanning/tyrosine kinase receptors
(Ullrich et al, 1985, *Nature*, 313:756-761; Ullrich et
al., 1986, *EMBO J*, 5:2503-2512). Other members of the
family, including relaxin, relaxin-3, INSL3, INSL4,
INSL5, and INSL6, may all signal through GPCRs (see Hsu
et al., 2003, *Trends Endocrinol. Metab.*, 14:303-309).
Based on the sequence/structure difference and the two
different ways that these peptides signal, it is believed
that relaxin, relaxin-3, INSL3, INSL4, INSL5, and INSL6
define a relaxin subfamily distinct from insulin and
IGFs.

The results from the radioligand binding assays and
the functional assays indicated that chimeras including
R_3/R_1, R_3/R_2, R_3/I_3 have substantially similar agonist
properties to that of the relaxin-3 wild type peptide in
terms of their ligand activity for GPCR135, GPCR142, and
LGR7. The relaxin-3 B-chain alone is sufficient to
activate GPCR135 and GPCR142, suggesting that the B-chain
of relaxin-3 may contain the necessary amino acid
residues that directly interact with GPCR135 or GPCR142.
With the addition of A-chain from other members of the
family to the relaxin-3 B-chain, it was anticipated that
some of the chimeric peptides would display a high
affinity for GPCR135 and GPCR142. Since relaxin-1,
relaxin-2, relaxin-3, and INSL3 are natural ligands for
either LGR7 (Hsu et al., 2002, *Science*, 295:671-674; Sudo
et al., 2003, *J. Biol. Chem.*, 278:7855-7862), LGR8
(Kumagai et al., 2002, *J. Biol. Chem.*, 277:31283-31286;
Bogatcheva, et al., 2003, *Mol. Endocrinol.*, 17:2639-
2646), or both (Hsu et al., 2002, *Science*, 295:671-674),
as was expected, R_3/R_1, R_3/R_2, R_3/I_3 displayed a very
similar pharmacological profile to relaxin-3 (i.e.,
potent ligands for GPCR135, GPCR142, and LGR7). It is

also noted that although relaxin-3 is not a ligand for
LGR8 (Sudo, et al., 2003, *J. Biol. Chem.*, 278:7855-7862),
R_3/R_1, R_3/R_2, and R_3/I_3 have demonstrated ligand activities
for LGR8, albeit with very low potency. Since those
chimeras have A-chains either from relaxin-1, relaxin-2,
or INSL3, which are natural ligands for LGR8, it is
possible that the A-chains in those chimeras interact
with and stimulate LGR8. It has been demonstrated that
the relaxin-3 B-chain alone is not sufficient to activate
LGR7 or LGR8. However, the B-chains of relaxin and INSL3
have been reported to play important roles for relaxin
and INSL3 to bind to and activate LGR7 or LGR8
(Bullesbach et al, 1992, *J. Biol. Chem.*, 267:22957-22960;
Bullesbach et al., 1999, *Biochemistry*, 38:3073-3078; Tan
et al., 2002, *Eur. J. Pharmacol.*, 457:153-160). These
results suggest that LGR7 and LGR8 interact with both the
B-chain and A-chain of their ligands. In addition, the
results herein reflect a trend in which chimeras with
relaxin-3 B-chain and an A-chain from a natural ligand
for LGR7 or LGR8 tended to have higher affinity for LGR7
or to be active for LGR8. Conversely, relaxin-3 chimeric
peptides with A-chains from members that are not ligands
for LGR7 or LGR8 have lower or no affinity for LGR7 or
LGR8, although they may still retain high affinity for
GPCR135 and GPCR142.

From the results disclosed herein, a highly
selective chimera for GPCR135/GPCR142 versus LGR7 and
LGR8 is R_3/I_5. R_3/I_5 retains high affinity for
GPCR135/GPCR142 but displays low affinity for LGR7 and is
inactive towards LGR8, indicating that INSL5 may not be a
natural ligand for LGR7 or LGR8. The chimeric peptide
R_3/I_6 showed reasonably high affinity (10 -15 nM) for
GPCR135/GPCR142 and LGR7, but is inactive for LGR8,
suggesting that the cognate receptor for INSL6 may be a

close neighbor of LGR7; however, it does not appear to be LGR8. Chimeric peptide R_3/I_4 had no activity for LGR7 and LGR8, suggesting that INSL4 is not a ligand for LGR7 or LGR8. The R_3/I_4 chimeric peptide demonstrated no improved activity compared to the relaxin-3 B-chain alone in terms of ligand activity for GPCR135 and GPCR142. Given that INSL4 is only found in human (Koman, et al., 1996, *J. Biol. Chem.*, 271:20238-20241; Hsu et al., 2003, *Trends. Endocrinol. Metab.*, 14:303-309), INSL4 may be a unique member of the relaxin subfamily and has distinct structure; therefore the A-chain of INSL4 was not able to present the B-chain of relaxin-3 in the R_3/I_4 chimera in an appropriate configuration to interact with GPCR135/GPCR142 at high affinity.

Table 1. IC_{50}[a] (nM) values for relaxin-3 chimeras to binding GPCR135, GPCR142, LGR7 and LGR8

	GPCR135	GPCR142	LGR7	LGR8
R_3/R_1	2.9 ± 0.35	2.6 ± 0.31	5.1 ± 0.78	> 1000
R_3/R_2	1.2 ± 0.27	1.8 ± 0.36	1.5 ± 0.37	615 ± 55
R_3/I_3	2.3 ± 0.32	3.5 ± 0.47	2.3 ± 0.35	475 ± 32
R_3/I_4	> 1000	> 1000	ND[b]	ND
R_3/I_5	0.68 ± 0.13	1.4 ± 0.35	724 ± 83	ND
R_3/I_6	9.5 ± 1.7	11.3 ± 2.3	12.9 ± 1.9	ND

[a] IC_{50} values (mean ± SEM, n = 3) were ligand concentrations that inhibit 50% of the maximum specific bindings

[b] ND denotes no activity was detected

Table 2. EC_{50} (nM) values of relaxin-3 chimeras to activate GPCR135, GPCR142, LGR7 and LGR8

	GPCR135[a]	GPCR142[a]	LGR7[b]	LGR8[b]
R_3/R_1	1.8 ± 0.27	2.2 ± 0.31	4.5 ± 0.51	>1000
R_3/R_2	1.1 ± 0.14	1.4 ± 0.26	1.1 ± 0.16	575 ± 65
R_3/I_3	2.2 ± 0.31	2.9 ± 0.28	2.5 ± 0.32	258 ± 42
R_3/I_4	>1000	>1000	ND[c]	ND
R_3/I_5	0.45 ± 0.1	0.91 ± 0.21	412 ± 74	ND
R_3/I_6	8.4 ± 1.6	11.2 ± 2.5	13.7 ± 1.9	ND

[a] EC_{50} values (mean ± SEM, n = 3) for GPCR135 and GPCR142 were ligand concentrations that achieve 50% of the maximum ligand induced inhibition of forskolin stimulated β-galactosidase expression

[b] EC_{50} values (mean ± SEM, n = 3) for LGR7 and LGR8 were ligand concentrations that induced 50 % of the maximum ligand stimulated β-galactosidase expression

[c] ND denotes no activity was detected

EXAMPLE 6

Materials and Methods

Production and Purification of Relaxin Family Proteins

The relaxin-3/INSL5 (R3/I5) chimeric polypeptide expression construct described in Example 1 was cotransfected into HEK293 cell with a furin expression vector (Liu et al., 2003, *J. Biol. Chem.*, 278:50754-50764) using LipofectaAmine (Invitrogen) as the transfection reagent. The transfected HEK293 cells were selected under G418 (400 mg/L) and the stable transformants were analyzed for R3/I5 fusion protein production by Western Blotting using an anti-FLAG antibody. A clone with high level of R3/I5 expression as well as thorough processing of the pre-propolypeptide was grown in a 1 liter fermenter. The N-terminal FLAG-tagged R3/I5 chimeric peptide was then purified from the medium using an anti-FLAG column as described Liu et al., 2003, *Journal of Biological Chemistry*, 278:50754-50764. Once prepared, stable cells expressing the protein of interest were inoculated into a CellMax Capillary Cell Culture System with a 50 to 150 kDa MWCO membrane and maintained using a CellMax Quad Cell Culture System (Spectrum Laboratories, Rancho Dominguez, CA). The transformed HEK-293 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10 mM HEPES pH 7.4, 1X non-essential amino acid mix, 1 mM sodium pyruvate, 2 mM glutamine, 50 u/ml penicillin and 50 μg/ml streptomycin (all from Hyclone, Logan Utah). Since the molecular weight cutoff of the membranes in the bioreactor was much larger than the molecular radius of

the products, the medium from the supply bottle was
retained for purification and the cell chamber was left
undisturbed. The Bioreactor apparatus was placed in a
37°C incubator with the CO_2 level set at 6%.

Enriched medium from the bioreactor was passed over
an affinity column containing anti-FLAG M2 agarose
(Sigma, St Louis, MO). The column volume and geometry
was optimized for the scale of each purification. The
enriched medium was passed through the column by gravity,
washed with 20 column volumes of Dulbecco's phosphate
buffered saline (Hyclone, Logan, UT) and eluted with 100
mM Glycine HCl pH 3.5. The column was eluted in 1 column
volume fractions into test tubes containing equimolar
Tris HCl pH 7.5, which immediately pH neutralized the
product. Removal of the FLAG tag was accomplished using
10 units/ml enterokinase (Novagen, Madison, WI) incubated
overnight at 37°C.

Final purification of the cleaved, affinity purified
protein was achieved by HPLC. The protein was loaded
onto a 10 mm id x 250 mm C18 column (Vydac, Hesperia, CA)
coupled to a Akta chromatograph (Amersham Biosciences,
Piscataway, NJ). The mobile phase was 0.1%
trifluoroacetic acid, which flowed through the column at
3 ml/min. The protein was eluted from the column with a
acetonitrile gradient. Aliquots of the purified fraction
were lyophilized and stored at -80°C.

Preparation of Radioligands

Radioiodination of R3 and the R3/I5 chimera was
accomplished as previously described (Vale, et al., 1983,
J. Cell. Biochem., 22(2):99-109). Briefly, 1 mCi Na[^{125}I]
was reacted with 10 µg peptide and 10 µg Chloramine T in
70 µl 100 mM KPO4 pH 7.4 for 30 seconds at room
temperature. The reaction was quenched with 50 µl of 10%

w/v bovine serum albumin, then pre-purified using Waters
Maxi-Clean C18 cartridges (Alltech, San Jose, CA). The
hydrophilic phase for the reversed phase separations
(prepurification and gradient HPLC) was 0.1% TFA and the
eluent was 0.1% TFA in acetonitrile. After removing the
acetonitrile from the Maxi-Clean eluent by partial
lyophilization the labeled compound was further purified
by gradient HPLC using a Vydac (Hesperia, CA) analytical
C18 HPLC column. A gradient of 2% CH_3CN/minute was used
and fractions were collected every 0.75 minute. Baseline
separation was achieved between the iodinated product and
reagents in each case.

Radioligand competition binding assays

The assays were performed using [125I] human R3 and
[125I] human R2 (each at a specific activity of 2200
Ci/mmol) as previously described in Liu et al., 2003,
Journal of Biological Chemistry 278:50754-50764. Cell
membranes from COS-7 cells transiently expressing rat
GPCR135 or LGR7 were incubated with [125I] human R3 or
[125I] human relaxin-2 (R2) (final concentration 100 pM)
in the presence of various concentrations of human
relaxin (R1), R3 and R3/I5. The reaction was incubated at
room temperature for 1 hour and the membranes were
filtered through the 96-well GFC plates (Packard
Instrument Co., Meriden, CT) using a cell harvester
(Packard Instrument Co). The filter was washed with the
ice cold washing buffer (50 mM Tris-HCl, pH 7.5). Fifty
µl of Microscint 40 (Packard Instrument Co) was added to
each well and the plates were counted on a Topcount NTX
(Packard Instrument Co). Non-specific binding was
determined in the presence of 100 nM R3 for GPCR135 and
100 nM relaxin for LGR7. The results were analyzed using
the PRISM software (GraphPad, San Diego, CA).

Animals

Adult male Sprague-Dawley rats (150-200 grams) were used in all experiments. They were housed two per cage, in a 12:12 h light:dark cycle and had free access to food and water. All the experiments described in this study have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institute of Health.

Tissue preparation for in situ hybridization and receptor autoradiography

Rats were asphyxiated by carbon dioxide. Brains were immediately removed from the skull and rapidly frozen in dry-ice. Twenty-micron-thick frontal sections were cut using a Cryostat-microtome (Microm HM505E, Mikron, San Diego, CA) and thaw-mounted on adhesive microscope slides (Superfrost⁺ Plus, VWR). The sections were kept at -70°C until use. The sectioning protocol for the regional distribution study included series of coronal sections covering the entire brain. Adjacent sections at the level of olfactory bulb, cortex and superior colliculus were used to generate concentration binding curves.

In Situ Hybridization

In situ hybridization was performed using a protocol taken from Simmons et al. (Simmons et al., 1989, *Journal of Histotechnology* 12(3):169-181) A full-length probe was prepared from the full-length rat GPCR135 cDNA (GenBank accession number AY394501) cloned into the pCI-Neo plasmid. Transcription was accomplished using the Promega (Madison, WI) Riboprobe Combination T3/T7 kit and [^{35}S]-UTP from Perkin Elmer (Boston, MA). Twenty micron

sections prepared as described above were fixed onto Superfrost + slides using 40 g/l paraformaldehyde buffered to pH 9.5 with sodium tetraborate and sodium hydroxide. Fixation was allowed to proceed for 30 minutes at 4°C, after which the slides were washed in Dulbecco's PBS, treated with 3 μg/ml proteinase K (Sigma-Aldrich, St Louis, MO), acetylated with 1:400 acetic anhydride in 0.1M triethanolamine at pH 8.0, rinsed in 2X SSC and dehydrated through a series of alcohol baths. After drying the tissue for 2 hours in a vacuum dessicator at room temperature the hybridization solution (Simmons et al., 1989, *Journal of Histotechnology* 12(3):169-181) was applied. Glass coverslips were applied to the slides, air bubbles were removed and the outside of the slides was sealed with DPX mountant (Fluka, Milwaukee, WI) and the slides were incubated at 60°C overnight. The cover slips were then removed in 4X SSC and the slides washed 4 times in 4X SSC baths. The slides were then treated with 20 μg/ml RNAse A (Sigma), rinsed and washed in baths of increasing stringency (0.5X SSC and 0.1X SSC). The stringency wash was 0.2X SSC with 50% formamide for 30 minutes at 65°C. Following the stringency wash the slides were washed in 0.1X SSC and dehydrated through an ethanol series. The slides were then dried in a vacuum dessicator and exposed to autoradiography film (Kodak Biomax MR, Rochester NY) for 4 weeks, then processed using a Konica SRY-101A film processor (Konica Medical Corp., Wayne NJ).

Receptor autoradiography on rat brain sections

Sections were thawed and dried under a cold air stream and then pre-incubated for 15 min. at room temperature in incubation buffer (20 mM HEPES, pH 7.4, 120 mM NaCl$_2$, 0.22 mM KH$_2$PO$_4$, 1.3 mM CaCl$_2$, 0.8 mM MgSO$_4$)

by immersing sections in a 400 ml jar. Sections were dried again under a cold air stream and incubated for 60 min. with 7 pM [^{125}I]-R3/I5 (specific activity, 2200 Ci/mmole) in incubation buffer containing 0.5% BSA and protease inhibitor cocktail. Inhibition of [^{125}I]-R3/I5 binding sites by human R3 or relaxin were performed in several brain regions (olfactory bulb, cortex and superior colliculus). Nonspecific binding was determined in the presence of 100 nM of human R3. After incubation, the excess radioligand was washed off by immersing the slides in the jar containing incubation buffer at 4°C (3 times 10 min.) followed by a quick immersion in water. Sections were dried and exposed to Fujifilm Imaging plates (BAS-MS 2025) for 48 hrs. The imaging plates were scanned using the Fuji Bio-Imaging Analyzer System (BAS-5000, Fuji Medical System, CT) and visualized using ImageGauge V3.12 software (Fuji Medical System, CT).

Cresyl-violet staining was performed on adjacent sections and the regions were identified using the rat brain atlas of Paxinos and Watson (Paxinos et al., 1998, <u>The Rat Brain in Stereotaxic Coordinates</u>, New York, Academic Press (publisher)). For illustration purposes, sections were exposed to autoradiography film ((Kodak Omat AR, Rochester NY). After a 4-week exposure, they were automatically developed and fixed in a Konica SRY-101A machine (Konica Medical Corp., Wayne NJ). Adobe Photoshop 7.0 and Illustrator 9.0 were used for preparation of the figures.

Sigmoidal competition binding curves were fitted by non-linear regression analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). The IC_{50} values were derived from the curve calculation. The IC_{50} is the concentration producing 50% inhibition of specific radioligand binding.

Results

Radioligand competition binding analysis of recombinant rat GPCR135 and LGR7 receptors

Competition binding experiments were on membrane preparations of recombinant rat GPCR135 and LGR7 receptors to compare the affinities of R3/I5 for these 2 receptors using $[^{125}I]$-relaxin-3 (R3) and $[^{125}I]$relaxin-2 (R2) as tracers (Figs. 7A and 7B, respectively). R3/I5 was found to be 800 fold selective for the rat GPCR135 (IC_{50} = 0.5 nM) over the rat LGR7 (IC_{50} > 400 nM). Relaxin displayed only weak affinity for $[^{125}I]$R3 binding sites in cells expressing rat GPCR135 receptors (IC_{50} > 1 μM) but potently competed for $[^{125}I]$R2 binding in cells expressing rat LGR7 receptors (IC_{50} = 200 pM).

Pharmacological characterization of $[^{125}I]$-R3/I5 binding sites on rat brain sections

Rat brain slides were incubated with 7 pM $[^{125}I]$-R3/I5 Fig. 8A). Pharmacological characterization of these binding sites was performed in several brain regions where high binding densities were observed (i.e. olfactory bulb, cortex, and superior colliculus) by measuring the potency of R3 or relaxin to inhibit specific $[^{125}I]$-R3/I5 binding. Representative images at the level of the superior colliculus in the presence of 10 nM of R3 or 100 nM relaxin-1 are shown in Figs. 8B and 8C, respectively.

The corresponding competition binding curve is shown in Fig 9. Affinities (IC_{50} values) of R3 for $[^{125}I]$-R3/I5 binding sites in various brain regions are given in Table

3. Starting at low concentration, R3 displaced all the [^{125}I]-R3/I5 binding sites in all regions investigated. Full displacement of [^{125}I]-R3/I5 binding sites by R3 was observed at 10 nM (Figs. 8A and 8B). IC_{50} values ranged from 0.3 to 0.4 nM (Table 3). Hill slope values were not statistically different from unity.

In contrast to relaxin-3, in all the regions investigated, relaxin did not displace any significant amount of [^{125}I]-R3/I5 binding sites at concentrations of up to 100 nM (Fig.8C).

Anatomical localization of GPCR135 mRNA and [^{125}I]R3/I5 binding sites in rat brain

The anatomical distribution of GPCR135 mRNA receptor and [^{125}I]R3/I5 (7 pM) binding sites was studied in detail throughout the rat brain using in situ hybridization and receptor autoradiography on adjacent coronal sections. In general, the distribution pattern of GPCR135 mRNA receptor overlaps with the distribution of [^{125}I]R3/I5 binding sites. A semiquantitative overview is presented in Table 4. Autoradiograms showing the rostrocaudal distribution of GPCR135 mRNA and [^{125}I]R3/I5 binding sites are shown in Fig. 10 (front and mid brain) and Fig. 11 (hind brain). Incubation with the sense probe did not yield any hybridization signal. Non-specific binding was defined in the presence of 100 nM R3 and was found to be low (Figs. 10A'' to 10F'' and 11A'' to 11F'').

Specific hybridization signals and binding sites were restricted to distinct areas of the brain, including the olfactory system, septum, cortex, habenula, hypothalamus, amygdala, thalamus, hippocampal formation and several brain stem regions.

High densities of both mRNA and receptor binding sites were observed in the olfactory bulb (Fig. 10A,

10A') and olfactory nucleus (Fig. 10B, 10B'). In the
septum (lateral part), moderate densities of mRNA were
detected whereas only very low levels of binding sites
were detectable (Fig. 10C, 10C'). Within the
hypothalamus, mRNA and binding sites were restricted to
the paraventricular nucleus (Fig. 10D, 10D', 10E, 10E')
and supraoptic nucleus (Fig. 10D, 10D'). In addition,
GPCR135 mRNA was detected in lateral hypothalamus (Fig.
10D). In the latter region, only low densities of
binding sites were detectable (Fig. 10D').

Moderate to high densities of both GPCR135 mRNA and
binding sites were detected in the thalamus, mainly in
the paraventricular nucleus and centro medial nucleus
(Fig. 10E, 10E'). Within the hippocampal formation,
GPCR135 mRNA labeling was restricted to the CA3 field and
dentate gyrus (Fig. 10F). Only low densities of binding
sites were detectable in these hippocampal regions (Fig.
10F). In the habenula, moderate to high densities of
both hybridization signal and binding sites were detected
(Fig. 10F, 10F').

GPCR135 mRNA and binding sites were also present in
the amygdala, with dense labeling the medial, central,
lateral nuclei (Fig 10E, 10E', 10F, 10F') and within the
bed nucleus of the stria terminalis (Fig. 10D, 10D').
Strong labeling of both mRNA and binding sites was also
observed in the amygdalohippocampal area (Fig. 11A,
11A').

Strong labeling of both GPCR135 mRNA and binding
sites was observed in the superior colliculus (Fig. 11A,
11A', 11B, 11B') and interpeduncular nucleus (Fig. 11B,
11B').

Within the dorsal part of the dorsal raphe,
periaqueductal gray area and inferior colliculus,

moderate to low densities of GPCR135 mRNA and binding
sites were detected (Fig. 11C, 11C', 11D, 11D').

Through the cortical mantle higher densities of
binding sites of GPCR135 were observed compared to the
mRNA levels (Figs. 10 and 11). Moderate densities of
binding sites were observed in motor (Fig. 10D'),
somatosensory (Fig. 10D'), temporal and visual (Fig.
11C') cortices.

Finally, both GPCR135 mRNA and binding sites were
found in the nucleus incertus (Fig. 11E, 11E'), spinal
trigeminal tract and nucleus of solitary tract (Fig. 11F,
11F').

Table 3: Potency of R3 (IC_{50}, mean ± S.E.M., n = 3) for
inhibition of [^{125}I]R3/I5 binding sites in several rat
brain regions measured using quantitative receptor
autoradiography.

	[^{125}I]R3/I5
Olfactory bulb	0.3 ± 0.1
Cortex	0.4 ± 0.2
Superior colliculus	0.3 ± 0.1

Table 4: Distribution of GPCR135 mRNA and [^{125}I]R3/I5
binding sites in rat brain (adjacent sections) determined
using *in situ* hybridization and receptor autoradiography.
Semi-quantitative values are given: - not detected, + low
density, ++ moderate density, +++ high density (n = 3).

	GPCR135 mRNA	[^{125}I]R3/I5

Olfactory System		
Olfactory bulb	+++	+++
Anterior olfactory nucleus	+++	+++
Septal region		
Lateral	++	+
Cortex		
Motor	+	++
Somatosensory	+	++
Piriform	+	++
Temporal	+	++
Visual	+	++
Habenula		
Medial	++	++
Hypothalamus		
Paraventricular nucleus	+++	++
Supraoptic nucleus	+++	++
Lateral nucleus	++	-
Amygdaloid body		
Central nucleus	++	++
Lateral nucleus	++	++
Medial nucleus	++	++
Amygdalohippocampal area	+++	+++
Bed nucleus stria terminalis	++	++

Thalamus		
Paraventricular	++	++
Centro medial	++	++
Centro lateral	++	++
Hippocampal formation		
CA3 field	++	+
Dentate gyrus	++	+
Brain stem		
Superior colliculus	+++	+++
Inferior colliculus	++	++
Interpeduncular nucleus	+++	+++
Dorsal raphe, dorsal part	++	+
Periaqueductal gray area	++	++
Nucleus incertus	++	++
Spinal trigeminal tract	+	++

Discussion of Results of Example 6

Radiolabeling of the R3/I5 chimera yielded a tracer that was important to successful binding autoradiography studies. Initial efforts to map GPCR135 binding sites with [^{125}I]-R3 were unsuccessful because R3 is essentially uncharged and hydrophobic. The level of non-specific binding attainable with [^{125}I]-R3 is sufficient for work with recombinant receptor systems; however the [^{125}I]-R3 tracer was unamenable to workable conditions for autoradiographic binding studies. Substituting the A

chain of insulin 5 in the R3 molecule imposes a net charge and reduces non-specific binding of the radioiodinated R3/I5 chimera (Figure 11).

GPCR135 binding sites in the rat central nervous system were selectively identified after binding of $[^{125}I]$-R3/I5 (at a concentration of 7 pM). $[^{125}I]$-R3/I5 binding was fully displaced by 10 nM R3, but not by 100 nM relaxin, consistent with the pharmacology of GPCR135 (Figs. 8A, 8B, and 8C and Table 3). In the rat, the only receptor other than GPCR135 that is known to bind R3 is LGR7. However, LGR7 lacks significant affinity for R3/I5 (Fig. 3C and 7B). Since GPCR142 is a pseudogene in the rat, the only known receptor able to yield the observed binding pattern is GPCR135.

The data show that the GPCR135 receptor density is most prominent in areas known for processing of sensory signals such as the olfactory bulb, sensory cortex, amygdala, thalamus, spinal trigeminal tract, inferior and superior colliculus. The level of mRNA expression observed was generally consistent in location and relative intensity with $[^{125}I]$-R3/I5 receptor binding autoradiography results. There were exceptions where GPCR135 mRNA expression was greater (i.e. the lateral septum, CA3 field and dentate gyrus) or less (i.e. cerebral cortical areas) than would be expected based on the binding autoradiography data; however the relative intensity of binding sites and expression were similar to each other.

A comparison of the results for R3/I5 binding to GPCR135 can be made with relaxin binding to LGR7 and LGR8 (Osheroff et al., 1991, *Prot. Natl. Acad. Sci. USA*, 88(15):6413-6417) to gain insights into signaling in this system. Since R3 originates almost exclusively from the nucleus incertus (with slight expression in the dentate

gyrus of the hippocampus (Burazin, et al., 2002, *J. Neurochem.*, 28:1553-1557; Liu et al., 2003,*J. Biol. Chem.*, 278:50754-50764; and Liu et al., 2003, *J. Biol. Chem.*, 278:50765-50770) and pathways of the nucleus incertus are well described (Goto et al., 2001, *J. Comp. Neurol.*, 438(1):86-122), the analysis can be extended to help identify which receptors (LGR7 versus GPCR135) in which brain areas respond to R3.

Many areas of GPCR135 expression and GPCR135-like receptor binding also bind relaxin (Osheroff et al., 1991, *Prot. Natl. Acad. Sci. USA*, 88(15):6413-6417). Use of a relaxin tracer should label LGR7 and LGR8 receptors, which are found in the brain and share a high affinity for relaxin (Hsu, 2003, *Trends Endocrinol. Metab.*, 14(7):303-309; Hsu et al., 2003, *Mol. Cell. Endocrinol.*, 202:165-170; and Hsu et al., 2002, *Science*, 295:671-674). Examples of areas apparently bearing both GPCR135 (as visualized with the specific tracers disclosed herein) and LGR7/8 (as shown with labeled relaxin (Osheroff et al., 1991, *Prot. Natl. Acad. Sci. USA*, 88(15):6413-6417) include the olfactory bulbs, anterior olfactory nucleus, paraventricular hypothalamic nucleus, supraoptic nucleus, thalamic nuclei (paraventricular and centromedial), the dentate gyrus of the hippocampus and the interpeduncular nucleus. GPCR135 expression and I5/R3 binding are found in the nucleus incertus, while relaxin binding is found in the dorsal tegmental nucleus. Dorsal tegmental relaxin binding observed by Osheroff and Phillips appears to be lateral to the GPCR135 expression/R3/I5 binding. Thus, R3/I5 binding sites and relaxin binding sites appear to be of sufficient proximity to allow paracrine, if not direct, communication.

Sites of R3/I5 binding in the CNS, which are similar to areas of GPCR135 expression, are found in a number of

areas lacking affinity for relaxin (Osheroff et al.,
1991, *Prot. Natl. Acad. Sci. USA*, 88(15):6413-6417).
Selective GPCR135 expression and I5/R3 binding can be
seen in the lateral septum, habenula, the superior
colliculus and inferior colliculus. Expression of
GPCR135 mRNA and I5/R3 binding sites in the amygdala
appear to be more extensive than has been observed for
relaxin binding sites, as GPCR135 sites/mRNA cover the
central, medial, and lateral amygdalar nuclei as well as
the bed nucleus of the stria terminalis. The GPCR135
coverage of the amygdala is so complete as to create a
semicircle surrounding the ventrolateral zones of the
thalamus. GPCR135 mRNA and I5/R3 binding sites are also
found in the CA3 field of the hippocampus, where relaxin
binding is lacking (Osheroff et al., 1991, *Prot. Natl.
Acad. Sci. USA*, 88(15):6413-6417).

Osheroff and Phillips found areas of relaxin binding
where neither GPCR135 expression nor GPCR135-like binding
was detected. The organum vasculosum of the lamina
terminalis, subfornical organ, arcuate hypothalamic
nucleus, mammillary peduncle, anterior pretectal nucleus
and the posterior hypothalamus all bind relaxin, but lack
indications of GPCR135.

Areas where GPCR135 mRNA and GPCR135-like binding
sites are detected share a common feature of involvement
in sensory perception. For instance, the olfactory bulbs
and olfactory nuclei, which showed signals for GPCR135
mRNA and GPCR135 binding sites, are early relays in the
transmission of olfactory signals. Similarly, GPCR135
mRNA and binding sites were detected in the visual and
somatosensory cortex, where sensory information is
processed or integrated for movement.

GPCR135 mRNA and I5/R3 binding sites were found in
some sensory areas of the brain lacking relaxin binding

sites (Osheroff et al., 1991, *Prot. Natl. Acad. Sci. USA*, 88(15):6413-6417). The superior colliculus, which bound R3/I5, is known for its involvement in visual attention and saccadic eye movements. Aural inputs pass through the inferior colliculus, which expresses GPCR135 mRNA and displays I5/R3 binding sites, on the way to the medial geniculate nucleus and the primary auditory cortex. Expression of GPCR135 mRNA and GPCR135-like binding sites were also detected in the lateral septum, which is involved in the processing of tactile stimuli (Adams, 2000,*Brain Research*, 858(1):177-180).

GPCR135 expression and I5/R3 binding in these areas suggest role(s) in the three key senses (visual, auditory and tactile). Notably, the superior colliculus, which displays particularly strong GPCR135-like receptor binding, has been linked to sensory and motor functions involving these 3 senses [King, 2004, *Curr. Biol.*, 14(9):R335-R338)].

Pathway tracing studies have mapped afferent and efferent projections of the nucleus incertus (Goto et al., 2001, *J. Comp. Neurol.*, 438(1):86-122) , which is the predominant source of R3 in the CNS. Comparing the results herein (Liu et al., 2003,*J. Biol. Chem.*, 278:50754-50764 and Liu et al., 2003, *J. Biol. Chem.*, 278:50765-50770) to those of Goto et al. (Goto et al., 2001, *J. Comp. Neurol.*, 438(1):86-122), R3 expressing cells are found in the nucleus incertus (NI) and in the adjacent central gray. The NI projects to a number of brain nuclei that express GPCR135 and have GPCR135-like binding sites. Ascending NI fibers pass through the interpeduncular nucleus, which expresses GPCR135 mRNA and has a significant concentration of binding sites. NI fibers passing through the interpeduncular nucleus project further to the thalamus, which also shows

pronounced GPCR135 mRNA expression and I5/R3 binding. Some NI fibers also project to the hypothalamus, where we show binding sites and mRNA expression. A sparse NI projection reaches the superior colliculus, which shows a high density of binding sites and strong GPCR135 mRNA expression. NI projections are also found in the amygdala, the supraoptic nucleus, dorsal raphe, medial habenula and cortical fields, all of which express GPCR135 mRNA and show binding sites. NI neurons project to the temporal pole of the dentate gyrus, which has been shown to exhibit light expression of R3 (Burazin et al., 2002, *J. Neurochem*, 82:1553-1557), weakly expresses of GPCR135 mRNA and has I5/R3 binding sites. In summary, nucleus incertus projections identified by Goto et al (Goto et al., 2001, *J. Comp. Neurol.*, 438(1):86-122), are generally consistent with our observations of GPCR135-like sites and mRNA.

As the primary source of R3 in the CNS, the NI is positioned to be central to physiological functions modulated by R3 and GPCR135. In addition to expressing R3, the NI strongly expresses mRNA for the type 1 CRF receptor (Potter et al., 1994, *Prot. Natl. Acad. Sci. USA*, 91:8777-8781). A number of areas that express GPCR135 such as the olfactory bulb, dentate gyrus, amygdala, superior colliculus, inferior colliculus, dorsal raphe, and solitary tract also express CRF-R1 and/or CRF-R2 mRNA (Potter et al., 1994, *Prot. Natl. Acad. Sci. USA*, 91:8777-8781; Chalmers et al., 1995, *J. Neurosci.*, 15(10):6340-6350 and Van Pett, 2000, *J. Comp. Neur.*, 428:191-212) GPCR135 mRNA expression and GPCR135-like binding sites are prominent in areas related to sensory processing, integration and motor control. It is believed that the NI is involved in extrapituitary actions of CRF (Goto et al., 2001, *J. Comp. Neurol.*,

438(1):86-122). Considering these factors, the NI may well be involved in regulating sensory perception and sensory-motor reflexes under stressful conditions.

Although various advantages and aspects of the invention have been illustrated above by reference to examples and preferred embodiments, it will be appreciated that the scope of the invention is defined not by the foregoing description, but by the following claims as properly construed under principles of patent law.

What is Claimed is:

1. A biologically active relaxin-3 chimeric polypeptide, comprising a relaxin-3 B-chain and a relaxin/insulin polypeptide family member A-chain.

2. A biologically active relaxin-3 chimeric polypeptide as defined in claim 1, wherein said relaxin/insulin polypeptide family member A-chain is selected from the group consisting of a relaxin-1 A-chain, a relaxin-2 A-chain, an insulin-like 3 A-chain, an insulin-like 4 A-chain, an insulin-like 5 A-chain, and an insulin-like 6 A-chain.

3. A biologically active relaxin-3 chimeric polypeptide as defined in claim 2, wherein said relaxin/insulin polypeptide family member A-chain is an insulin-like 5 A-chain.

4. A biologically active relaxin-3 chimeric polypeptide as defined in claim 3 having an amino acid sequence as set forth in SEQ ID NO:23.

5. A polynucleotide encoding a relaxin-3 chimeric pre-propolypeptide comprising a relaxin B-chain joined by a first peptide linkage to a relaxin C-chain, the C-chain further joined by a second peptide linkage to a relaxin/insulin family member A-chain, and a protease cleavage site inserted at at least one location selected from the second peptide linkage between the A-chain and C-chain and the first peptide linkage between the C-chain and the B-chain of the relaxin-3 chimeric pre-propolypeptide.

6.   A polynucleotide as defined in claim 5, wherein said A-chain is selected from the group consisting of a relaxin-1 A-chain, a relaxin-2 A-chain, an insulin-like 3 A-chain, an insulin-like 4 A-chain, an insulin-like 5 A-chain, and an insulin-like 6 A-chain.

7.   A polynucleotide as defined in claim 5, wherein said protease cleavage site is inserted at the peptide linkage joining the C-chain and A-chain.

8.   A polynucleotide as defined in claim 7, wherein said protease cleavage site is a furin cleavage site.

9.   A polynucleotide as defined in claim 5, having a nucleotide sequence selected from the group consisting of (i) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:7 and (ii) complements thereof which hybridize under stringent conditions to said polynucleotide.

10.   A pre-propolypeptide encoded by a polynucleotide as defined in claim 5.

11.   A pre-propolypeptide encoded by a polynucleotide as defined in claim 9.

12.   An expression vector suitable for expression in a recombinant host cell, said vector comprising a polynucleotide as defined in claim 9.

13.   A host cell comprising an expression vector as defined in claim 12.

14.   A receptor-ligand complex comprising a receptor component comprising GPCR135 or GPCR142, said receptor component bound to a ligand component comprising a biologically active relaxin-3 chimeric polypeptide as defined in claim 1.

15.   A receptor-ligand complex as defined in claim 14, wherein said biologically active relaxin-3 chimeric polypeptide has an amino acid sequence as set forth in SEQ ID NO:23 and bears a radioisotope label.

16.   A receptor-ligand complex as defined in claim 15, wherein said receptor component comprises GPCR135 or GPCR142 associated with isolated cell membranes or lipid vesicles.

17.   A receptor-ligand complex as defined in claim 15, wherein said receptor component and said ligand component are each in a substantially pure form.

18.   A receptor-ligand complex as defined in claim 15, wherein the receptor component comprises both GPCR135 and GPCR142.

19.   A process of producing a mature relaxin-3 chimeric polypeptide from a recombinant cell, comprising:

(a)   transforming or transfecting a host cell with a first expression vector as defined in claim 5 and a second vector expressing a protease for cleaving a relaxin-3 chimeric pre-propolypeptide at an inserted protease cleavage site; and

(b)   growing the host cell so that both the relaxin-3 chimeric pre-propolypeptide and the protease are expressed, whereby the protease cleaves a peptide linkage

at said inserted protease cleavage site on the relaxin-3 chimeric pre-propolypeptide yielding a mature relaxin-3 chimeric polypeptide.

20. A process as defined in claim 19, wherein the protease is furin and the protease cleavage site is a furin site.

21. A process as defined in claim 19, further comprising: expressing a receptor component on the cell surface of a host cell that has been transformed or transfected with an expression vector encoding GPCR135 or GPCR142; and complexing the receptor component with the relaxin-3 chimeric polypeptide.

22. A method of identifying a compound that modulates a biological activity of at least one receptor selected from the group consisting of GPCR135 and GPCR142, comprising the steps of:

(a) contacting (i) a test sample comprising a compound with (ii) an assay reagent comprising the at least one receptor and a relaxin-3 chimeric polypeptide as defined in claim 1;

(b) determining the biological activity of the at least one receptor after performing step (a); and

(c) comparing the biological activity determined in step (b) with a control measurement obtained by contacting a control sample not containing the compound with the assay reagent.

23. A method as defined in claim 22, wherein said at least one receptor is a component of a biological sample derived from a rat.

24. A method as defined in claim 22, wherein said at least one receptor is a component of a biological sample derived from a human.

25. A method as defined in claim 22, wherein said determining the biological activity comprises identifying the formation of a receptor-ligand complex.

26. A method as defined in claim 25, wherein the ligand component is radiolabeled, and said identifying the formation of a receptor-ligand complex comprises performing autoradiography.

27. A method as defined in claim 22, wherein said determining the biological activity comprises measuring a second messenger response.

28. A method as defined in claim 22, wherein said determining the biological activity comprises measuring a second messenger response by intracellular calcium ion concentration or intracellular cAMP concentration.

29. A method as defined in claim 22, wherein said at least one receptor is associated with isolated cell membranes from a GPCR135 host cell or a GPCR142 host cell, and said determining the biological activity of the at least one receptor comprises measuring the amount of protein phosphorylation of the isolated membranes using a γ-phosphate labeled GTP molecule selected from the group consisting of ^{35}S-GTPγS, ^{33}P-GTPγP, and ^{32}P-GTPγP.

30. A method as defined in claim 22, wherein the relaxin-3 chimeric polypeptide has an amino acid sequence as set forth in SEQ ID NO:23.

31.   A method of identifying a compound that binds
to at least one receptor selected from the group
consisting of GPCR135 and GPCR142, comprising the steps
of:

(a)   contacting the at least one receptor with a
test compound and with a labeled relaxin-3 chimeric
polypeptide as defined in claim 1;

(b)   determining the amount of the labeled relaxin-3
chimeric polypeptide that binds to the at least one
receptor; and

(c)   comparing the amount determined in step (b)
with a control measurement obtained by contacting the at
least one receptor with the labeled relaxin-3 chimeric
polypeptide in the absence of the test compound.

32.   A method as defined in claim 31, wherein said
at least one receptor is a component of a biological
sample derived from a rat.

33.   A method as defined in claim 31, wherein said
at least one receptor is a component of a biological
sample derived from a human.

34.   A method as defined in claim 31, wherein the
labeled relaxin-3 chimeric polypeptide comprises a
relaxin-3 B-chain and a relaxin/insulin polypeptide
family member A-chain, wherein said relaxin/insulin
polypeptide family member A-chain is selected from the
group consisting of a relaxin-1 A-chain, a relaxin-2 A-
chain, an insulin-like 3 A-chain, an insulin-like 4 A-
chain, an insulin-like 5 A-chain, and an insulin-like 6

A-chain, and wherein the labeled relaxin-3 chimeric polypeptide is labeled with a radiolabel.

35. A method as defined in claim 34, wherein the labeled chimeric relaxin-3 polypeptide has an amino acid sequence as set forth in SEQ ID NO:23.

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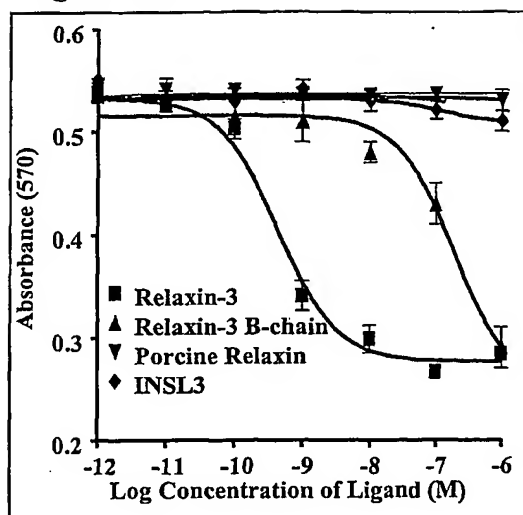
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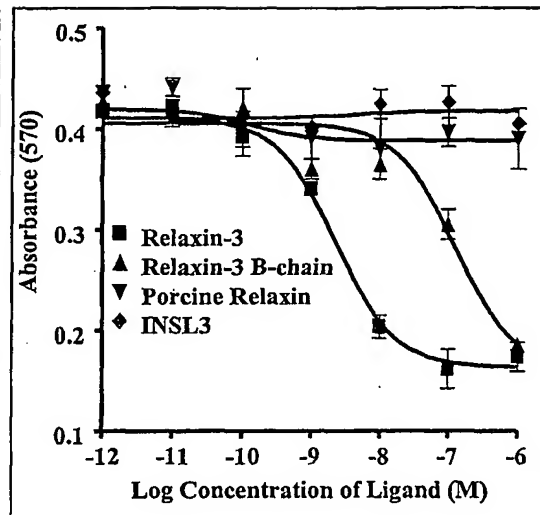
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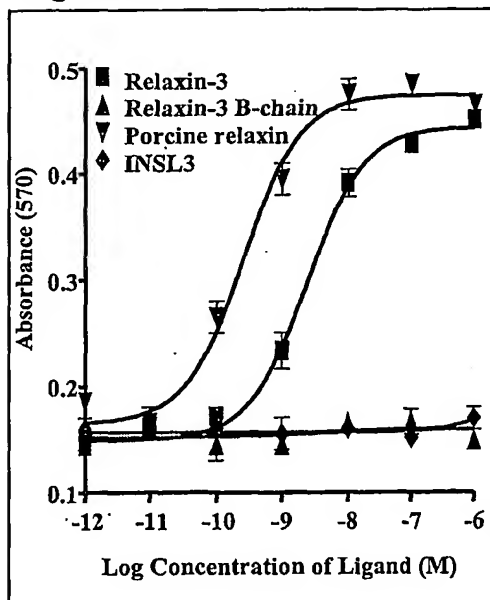
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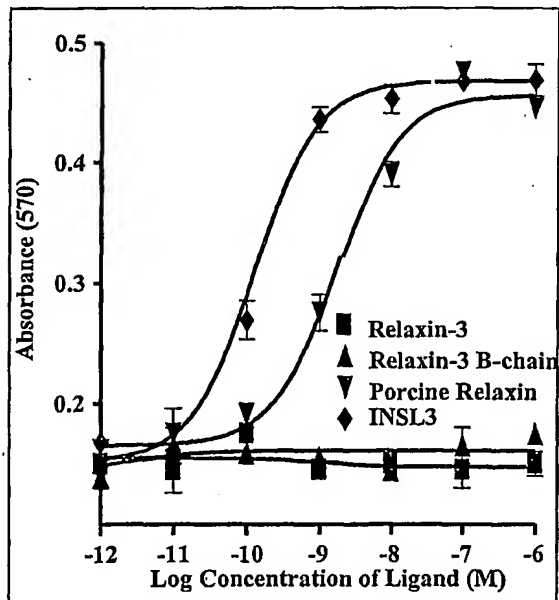
Figure 2

	B-chain	A-chain
Relaxin-3	RAAPYGVRLCGREFIRAVIFTCGGSRW	DVLAGLSSSCCKWGCSKSEISSLC
R3/R1	RAAPYGVRLCGREFIRAVIFTCGGSRW	RPYVALFEKCCLIGCTKRSLAKYC
R3/R2	RAAPYGVRLCGREFIRAVIFTCGGSRW	QLYSALANKCCHVGCTKRSLARFC
R3/I3	RAAPYGVRLCGREFIRAVIFTCGGSRW	ATNPARYCCLSGCTQQDLLTLC
R3/I4	RAAPYGVRLCGREFIRAVIFTCGGSRW	FDPFCCEVICDDGTSVKLC
R3/I5	RAAPYGVRLCGREFIRAVIFTCGGSRW	DLQTLCCTDGCSMTDLSALC
R3/I6	RAAPYGVRLCGREFIRAVIFTCGGSRW	GYSEKCCLTGCTKEELSIAC
R3/I	RAAPYGVRLCGREFIRAVIFTCGGSRW	GIVEQCCTSICSLYQLENYC
R3/A	RAAPYGVRLCGREFIRAVIFTCGGSRW	GLYENCCLSICDDYEIEKAC

Figure 3A

Figure 3B

Figure 3C
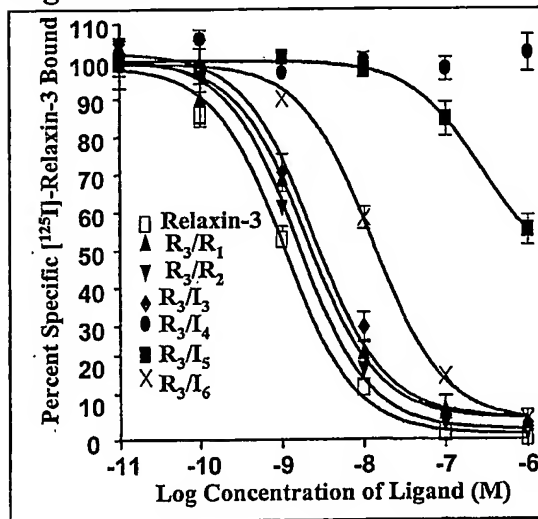
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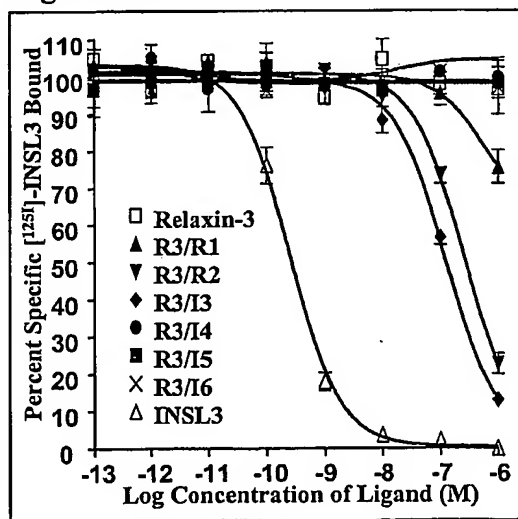
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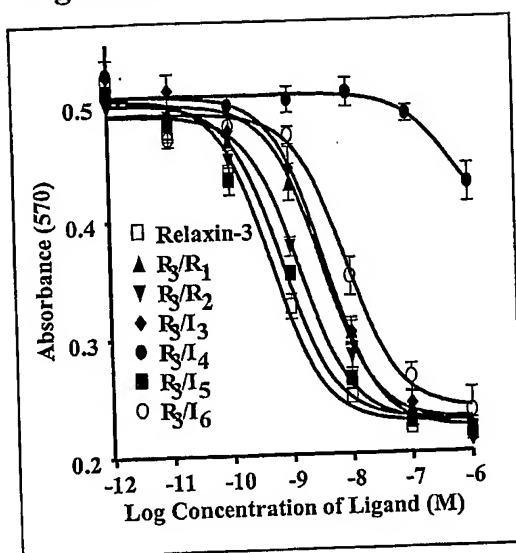
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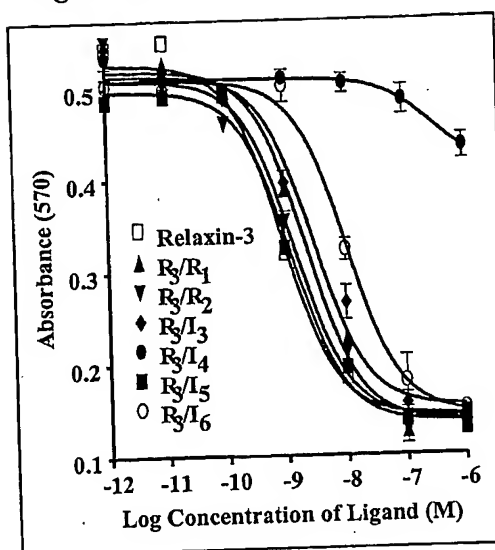
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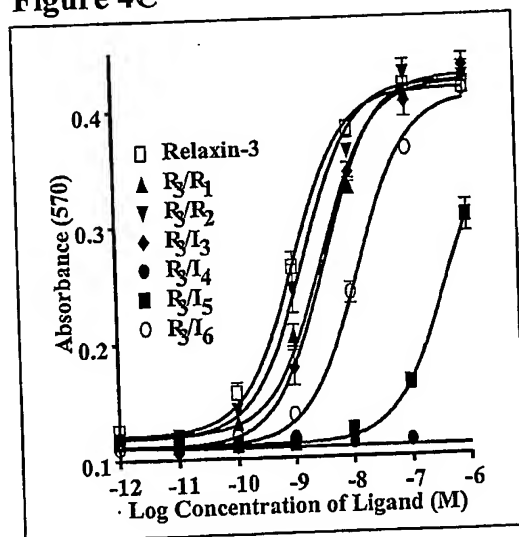
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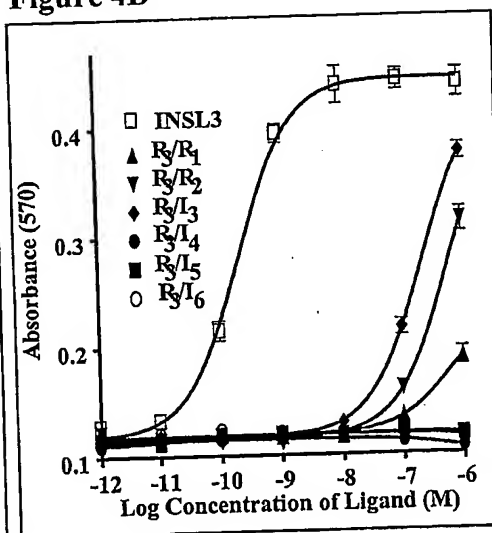
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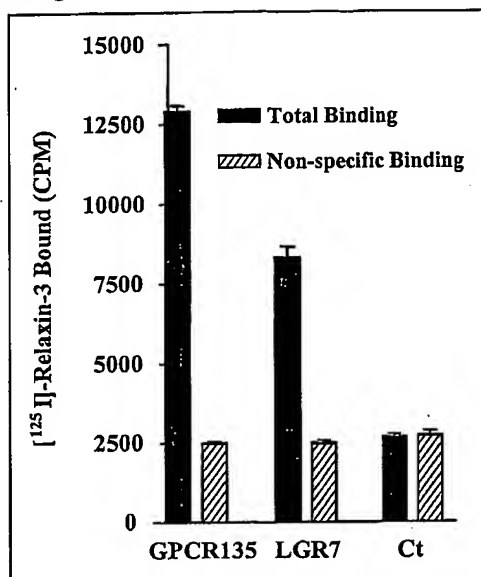
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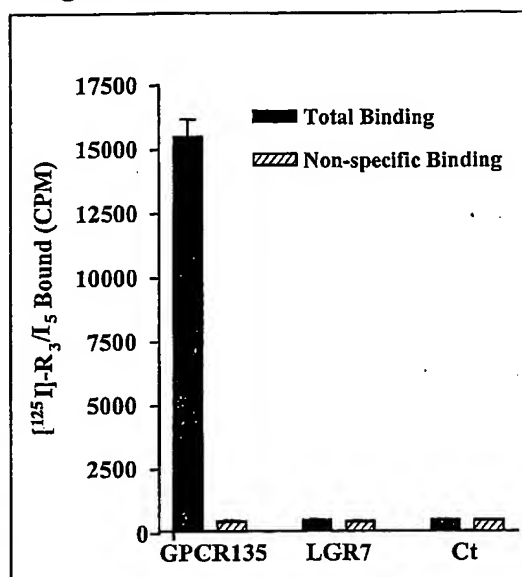
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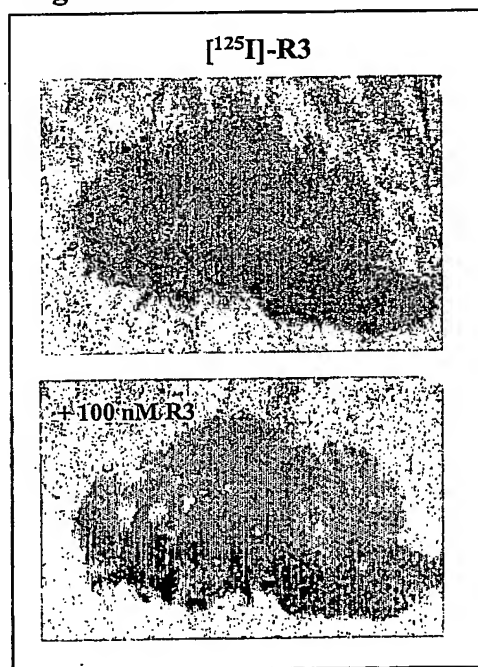
Figure 5D

Figure 6

B-chain

Relaxin-1:	KWKDDVIKLCGRELVRAQIAICGMSTWS
Relaxin-2:	DSWMEEVIKLCGRELVRAQIAICGMSTWS
Relaxin-3:	RAAPYGVRLCGREFIRAVIFTCGGSRW
INSL3:	PTPEMREKLCGHHFVRALVRVCGGPRWSTEA
*INSL4:	ESLAAELRGCGPRFGKHLLSYCPMPEK
*INSL5:	KESVRLCGLEYIRTVIYICASSRW
*INSL6:	ISSARKLCGRYLVKEIEKLCGHANWSQF
Insulin:	FVNQHLCGSHLVEALYLVCGERGFFYTPKT
IGF1:	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA
IGF2:	AYRPSETLCGGELVDTLQFVCGDRGFYFSRP-ASRVSRRSR---GIVEECCFRSCDLALLETYCATPAKSE

Relaxin Subfamily { Relaxin-1, Relaxin-2, Relaxin-3, INSL3, *INSL4, *INSL5, *INSL6 }

Insulin Subfamily { Insulin, IGF1, IGF2 }

A-chain

| RPYVALFEKCCLIGCTKRSLAKYC |
| QLYSALANKCCHVGCTKRSLARFC |
| DVLAGLSSSCCKWGCSKSEISSLC |
| NPARYCCLSGCTQQDLLTLC |
| SGRHRFDPFCCEVICDDGTSVKLCT |
| QDLQTLCCTDGCSMTDLSALC |
| GYSEKCCLTGCTKEELSIACLPYIDFKRL |
| GIVEQCCTSICSLYQLENYCN |

Figure 7A

R3	R3/I5	R3-F
7.6130e-010	5.8660e-010	7.5080e-009

Figure 7B

Figure 8

Figure 9
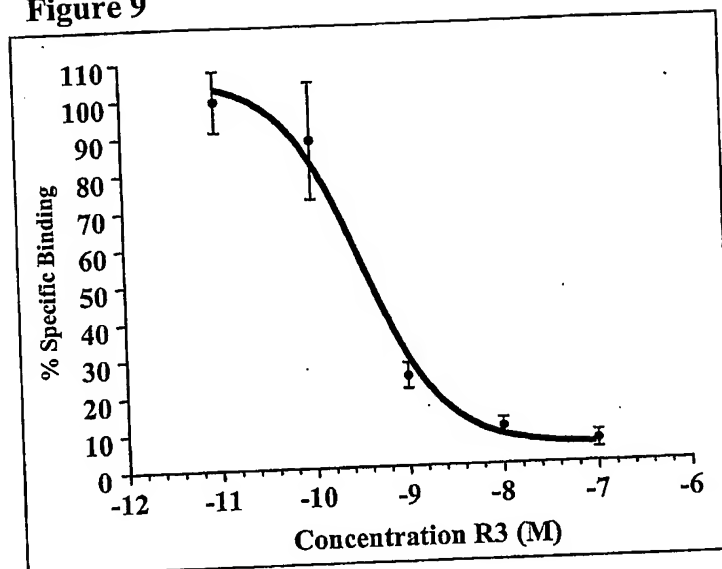
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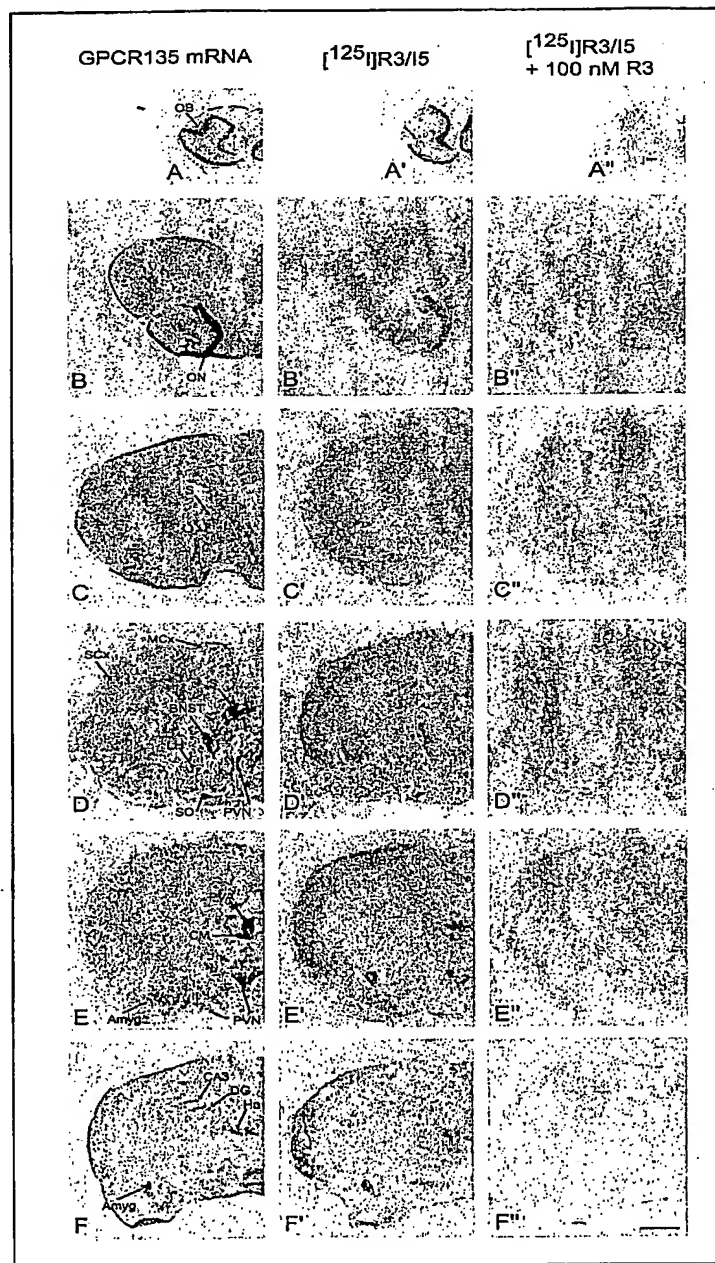
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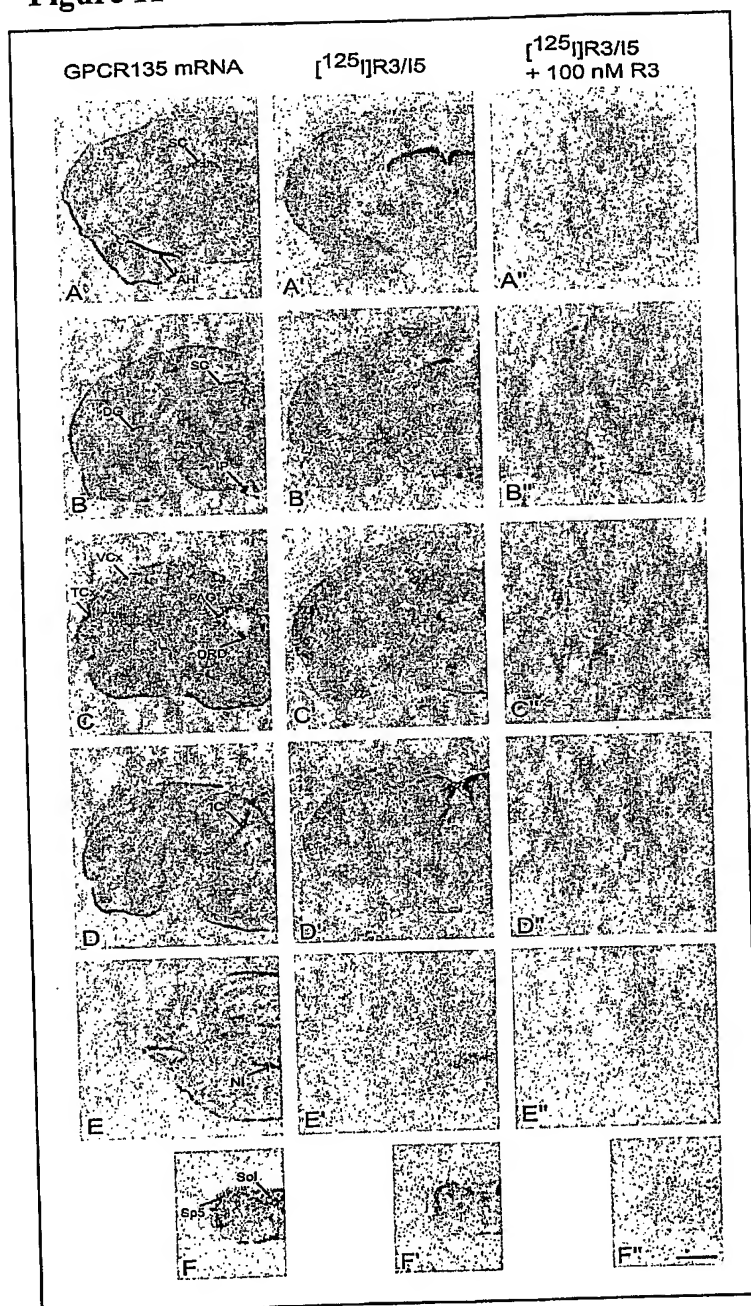
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<120> RELAXIN-3 CHIMERIC POLYPEPTIDES AND THEIR PREPARATION AND USE

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atgctgaccg cagcgttgct gagctgtgcc ctgctgctgg cactgcctgc cacgcgagga   60

gactacaagg acgacgatga caaggaattc gatgacgacg ataagcgggc agcgccttac   120

ggggtcaggc tttgcggccg agaattcatc cgagcagtca tcttcacctg cggggggctcc   180

cggtggagac gatcagacat cctggcccac gaggctatgg gagatacctt cccggatgca   240

gatgctgatg aagacagtct ggcaggcgag ctggatgagg ccatggggtc cagcgagtgg   300

ctggccctga ccaagtcacc ccaggccttt tacaggggggc gacccagctg gcaaggaacc   360

cctggggttc ttcggggccg acgacaactc tacagtgcat tggctaataa atgttgccat   420

gttggttgta ccaaaagatc tcttgctaga ttttgctga   459

<210>   5
<211>   447
<212>   DNA
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 3 chimeric prepropolypeptide cDNA

<400>   5
atgctgaccg cagcgttgct gagctgtgcc ctgctgctgg cactgcctgc cacgcgagga   60

gactacaagg acgacgatga caaggaattc gatgacgacg ataagcgggc agcgccttac   120

ggggtcaggc tttgcggccg agaattcatc cgagcagtca tcttcacctg cggggggctcc   180

cggtggagac gatcagacat cctggcccac gaggctatgg gagatacctt cccggatgca   240

gatgctgatg aagacagtct ggcaggcgag ctggatgagg ccatggggtc cagcgagtgg   300

ctggccctga ccaagtcacc ccaggccttt tacagggggc gacccagctg gcaaggaacc   360

cctggggttc ttcggggccg acgaaacccc gcccgctact gctgcctgtc cggctgcacc   420

cagcaggacc tgctgaccct gtgctga   447

<210> 6
<211> 444
<212> DNA
<213> Artificial

<220>
<223> Human Relaxin-3/Insulin-like 4 chimeric prepropolypeptide cDNA

<400> 6
atgctgaccg cagcgttgct gagctgtgcc ctgctgctgg cactgcctgc cacgcgagga   60

gactacaagg acgacgatga caaggaattc gatgacgacg ataagcgggc agcgccttac   120

ggggtcaggc tttgcggccg agaattcatc cgagcagtca tcttcacctg cggggggctcc   180

cggtggagac gatcagacat cctggcccac gaggctatgg gagatacctt cccggatgca   240

gatgctgatg aagacagtct ggcaggcgag ctggatgagg ccatggggtc cagcgagtgg   300

ctggccctga ccaagtcacc ccaggccttt tacagggggc gacccagctg gcaaggaacc   360

cctggggttc ttcggggccg acgattcgac cccttctgct gcgaggtgat ctgcgacgac   420

ggcacctccg tgaagctgtg ctga   444

<210> 7
<211> 450
<212> DNA
<213> Artificial

<220>
<223> Human Relaxin-3/Insulin-like 5 chimeric prepropolypeptide cDNA

<400> 7
atgctgaccg cagcgttgct gagctgtgcc ctgctgctgg cactgcctgc cacgcgagga   60

gactacaagg acgacgatga caaggaattc gatgacgacg ataagcgggc agcgccttac   120

ggggtcaggc tttgcggccg agaattcatc cgagcagtca tcttcacctg cggggggctcc   180

cggtggagac gatcagacat cctggcccac gaggctatgg gagatacctt cccggatgca   240

gatgctgatg aagacagtct ggcaggcgag ctggatgagg ccatggggtc cagcgagtgg   300

ctggccctga ccaagtcacc ccaggccttt tacagggggc gacccagctg gcaaggaacc   360

cctggggttc ttcggggccg acgacaagat ttacaaactt tgtgttgcac tgatggctgt   420

tccatgactg atttgagtgc tctttgctaa   450

<210> 8
<211> 447
<212> DNA
<213> Artificial

<220>
<223> Human Relaxin-3/Insulin-like 6 chimeric prepropolypeptide cDNA

<400> 8

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atgctgaccg cagcgttgct gagctgtgcc ctgctgctgg cactgcctgc cacgcgagga      60
gactacaagg acgacgatga caaggaattc gatgacgacg ataagcgggc agcgccttac     120
ggggtcaggc tttgcggccg agaattcatc cgagcagtca tcttcacctg cggggggctcc    180
cggtggagac gatcagacat cctggcccac gaggctatgg gagatacctt cccggatgca     240
gatgctgatg aagacagtct ggcaggcgag ctggatgagg ccatggggtc cagcgagtgg     300
ctggccctga ccaagtcacc ccaggccttt tacagggggc gacccagctg gcaaggaacc     360
cctggggttc ttcggggccg acgaggctac tccgagaagt gctgcctgac cggctgcacc     420
aaggaggagc tgtccatcgc ctgctga                                         447
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<210> 9
<211> 148
<212> PRT
<213> Artificial

<220>
<223> Human Relaxin-3/Random A chimeric prepropolypeptide

<400> 9

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Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1               5                  10                  15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Asp Asp
            20                  25                  30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
        35                  40                  45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50                  55                  60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65                  70                  75                  80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85                  90                  95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
                100                 105                 110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
            115                 120                 125

Gly Leu Tyr Glu Asn Cys Cys Leu Ser Ile Cys Asp Asp Tyr Glu Ile
        130                 135                 140

Glu Lys Ala Cys
145
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<210>   10
<211>   148
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin chimeric prepropolypeptide

<400>   10

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1               5                   10                  15


Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Asp Asp
            20                  25                  30


Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
            35                  40                  45


Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50                  55                  60


Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65                  70                  75                  80


Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85                  90                  95


Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
                100                 105                 110


Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
            115                 120                 125


Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
    130                 135                 140


Glu Asn Tyr Cys
145


<210>   11
<211>   152
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Relaxin-1 chimeric prepropolypeptide

<400>   11

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1               5                   10                  15


Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Asp Asp
            20                  25                  30
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Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
         35   40   45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50   55   60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65   70   75   80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85   90   95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100   105   110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115   120   125

Arg Pro Tyr Val Ala Leu Phe Glu Lys Cys Cys Leu Ile Gly Cys Thr
    130   135   140

Lys Arg Ser Leu Ala Lys Tyr Cys
145   150

<210>   12
<211>   152
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Relaxin-2 chimeric prepropolypeptide

<400>   12

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1   5   10   15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Lys Glu Phe Asp Asp
            20   25   30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
         35   40   45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50   55   60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65   70   75   80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85   90   95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100   105   110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115   120   125

Gln Leu Tyr Ser Ala Leu Ala Asn Lys Cys Cys His Val Gly Cys Thr
        130   135   140

Lys Arg Ser Leu Ala Arg Phe Cys
145   150

<210>   13
<211>   148
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 3 chimeric prepropolypeptide

<400>   13

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1   5   10   15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Lys Glu Phe Asp Asp
        20   25   30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
        35   40   45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50   55   60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65   70   75   80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85   90   95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100   105   110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115   120   125

Asn Pro Ala Arg Tyr Cys Cys Leu Ser Gly Cys Thr Gln Gln Asp Leu
    130   135   140

Leu Thr Leu Cys
145

<210>   14
<211>   147
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 4 chimeric prepropolypeptide

<400>   14

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1   5   10   15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Lys Glu Phe Asp Asp
            20   25   30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
        35   40   45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50   55   60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65   70   75   80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
            85   90   95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100   105   110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115   120   125

Phe Asp Pro Phe Cys Cys Glu Val Ile Cys Asp Asp Gly Thr Ser Val
    130   135   140

Lys Leu Cys
145

<210>   15
<211>   149
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 5 chimeric prepropolypeptide

<400>   15

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1   5   10   15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Asp Asp
            20   25   30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
        35   40   45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50   55   60

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```
Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65                  70              75                  80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85              90              95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100             105             110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115             120             125

Gln Asp Leu Gln Thr Leu Cys Cys Thr Asp Gly Cys Ser Met Thr Asp
    130             135             140

Leu Ser Ala Leu Cys
145
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<210> 16
<211> 148
<212> PRT
<213> Artificial

<220>
<223> Human Relaxin-3/Insulin-like 6 chimeric prepropolypeptide

<400> 16

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Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1               5               10                  15

Ala Thr Arg Gly Asp Tyr Lys Asp Asp Asp Lys Glu Phe Asp Asp
        20              25                  30

Asp Asp Lys Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu
        35              40              45

Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Arg Arg
    50              55              60

Ser Asp Ile Leu Ala His Glu Ala Met Gly Asp Thr Phe Pro Asp Ala
65                  70              75                  80

Asp Ala Asp Glu Asp Ser Leu Ala Gly Glu Leu Asp Glu Ala Met Gly
                85              90              95

Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln Ala Phe Tyr Arg
            100             105             110

Gly Arg Pro Ser Trp Gln Gly Thr Pro Gly Val Leu Arg Gly Arg Arg
        115             120             125

Gly Tyr Ser Glu Lys Cys Cys Leu Thr Gly Cys Thr Lys Glu Glu Leu
```

                    130   135   140

Ser Ile Ala Cys
145

<210>   17
<211>   47
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Random A chimeric mature polypeptide

<400>   17

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1   5   10   15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Gly Leu Tyr Glu Asn
            20   25   30

Cys Cys Leu Ser Ile Cys Asp Asp Tyr Glu Ile Glu Lys Ala Cys
        35   40   45

<210>   18
<211>   47
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin chimeric mature polypeptide

<400>   18

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1   5   10   15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Gly Ile Val Glu Gln
            20   25   30

Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys
        35   40   45

<210>   19
<211>   50
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Relaxin-1 chimeric mature polypeptide

<400>   19

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1   5   10   15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Pro Tyr Val Ala Leu
            20   25   30

```
Phe Glu Lys Cys Cys Leu Ile Gly Cys Thr Lys Arg Ser Leu Ala Lys
        35                  40              45

Tyr Cys
    50


<210>   20
<211>   51
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Relaixin-2 chimeric mature polypeptide

<400>   20

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1               5               10              15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Gln Leu Tyr Ser Ala
            20              25              30

Leu Ala Asn Lys Cys Cys His Val Gly Cys Thr Lys Arg Ser Leu Ala
        35                  40              45

Arg Phe Cys
        50


<210>   21
<211>   47
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 3 chimeric mature polypeptide

<400>   21

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1               5               10              15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Asn Pro Ala Arg Tyr
            20              25              30

Cys Cys Leu Ser Gly Cys Thr Gln Gln Asp Leu Leu Thr Leu Cys
        35                  40              45


<210>   22
<211>   46
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 4 chimeric mature polypeptide

<400>   22

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1               5               10              15
```

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Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Phe Asp Pro Phe Cys
                20   25   30

Cys Glu Val Ile Cys Asp Asp Gly Thr Ser Val Lys Leu Cys
                35   40   45

<210>   23
<211>   48
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 5 chimeric mature polypeptide

<400>   23

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1   5   10   15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Gln Asp Leu Gln Thr
                20   25   30

Leu Cys Cys Thr Asp Gly Cys Ser Met Thr Asp Leu Ser Ala Leu Cys
                35   40   45

<210>   24
<211>   47
<212>   PRT
<213>   Artificial

<220>
<223>   Human Relaxin-3/Insulin-like 6 chimeric mature polypeptide

<400>   24

Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1   5   10   15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Gly Tyr Ser Glu Lys
                20   25   30

Cys Cys Leu Thr Gly Cys Thr Lys Glu Glu Leu Ser Ile Ala Cys
                35   40   45

<210>   25
<211>   469
<212>   PRT
<213>   Homo sapiens

<400>   25

Met Gln Met Ala Asp Ala Ala Thr Ile Ala Thr Met Asn Lys Ala Ala
1   5   10   15

Gly Gly Asp Lys Leu Ala Glu Leu Phe Ser Leu Val Pro Asp Leu Leu
                20   25   30

Glu Ala Ala Asn Thr Ser Gly Asn Ala Ser Leu Gln Leu Pro Asp Leu

                    35   40   45

Trp Trp Glu Leu Gly Leu Glu Leu Pro Asp Gly Ala Pro Pro Gly His
    50   55   60

Pro Pro Gly Ser Gly Gly Ala Glu Ser Ala Asp Thr Glu Ala Arg Val
65   70   75   80

Arg Ile Leu Ile Ser Val Val Tyr Trp Val Cys Ala Leu Gly Leu
                85   90   95

Ala Gly Asn Leu Leu Val Leu Tyr Leu Met Lys Ser Met Gln Gly Trp
                100   105   110

Arg Lys Ser Ser Ile Asn Leu Phe Val Thr Asn Leu Ala Leu Thr Asp
            115   120   125

Phe Gln Phe Val Leu Thr Leu Pro Phe Trp Ala Val Glu Asn Ala Leu
    130   135   140

Asp Phe Lys Trp Pro Phe Gly Lys Ala Met Cys Lys Ile Val Ser Met
145   150   155   160

Val Thr Ser Met Asn Met Tyr Ala Ser Val Phe Phe Leu Thr Ala Met
                165   170   175

Ser Val Thr Arg Tyr His Ser Val Ala Ser Ala Leu Lys Ser His Arg
            180   185   190

Thr Arg Gly His Gly Arg Gly Asp Cys Cys Gly Arg Ser Leu Gly Asp
        195   200   205

Ser Cys Cys Phe Ser Ala Lys Ala Leu Cys Val Trp Ile Trp Ala Leu
    210   215   220

Ala Ala Leu Ala Ser Leu Pro Ser Ala Ile Phe Ser Thr Thr Val Lys
225   230   235   240

Val Met Gly Glu Glu Leu Cys Leu Val Arg Phe Pro Asp Lys Leu Leu
                245   250   255

Gly Arg Asp Arg Gln Phe Trp Leu Gly Leu Tyr His Ser Gln Lys Val
            260   265   270

Leu Leu Gly Phe Val Leu Pro Leu Gly Ile Ile Ile Leu Cys Tyr Leu
        275   280   285

Leu Leu Val Arg Phe Ile Ala Asp Arg Arg Ala Ala Gly Thr Lys Gly
    290   295   300

Gly Ala Ala Val Ala Gly Gly Arg Pro Thr Gly Ala Ser Ala Arg Arg
305   310   315   320
                            Page 13

Leu Ser Lys Val Thr Lys Ser Val Thr Ile Val Val Leu Ser Phe Phe
              325   330   335

Leu Cys Trp Leu Pro Asn Gln Ala Leu Thr Thr Trp Ser Ile Leu Ile
              340   345   350

Lys Phe Asn Ala Val Pro Phe Ser Gln Glu Tyr Phe Leu Cys Gln Val
              355   360   365

Tyr Ala Phe Pro Val Ser Val Cys Leu Ala His Ser Asn Ser Cys Leu
    370   375   380

Asn Pro Val Leu Tyr Cys Leu Val Arg Arg Glu Phe Arg Lys Ala Leu
385   390   395   400

Lys Ser Leu Leu Trp Arg Ile Ala Ser Pro Ser Ile Thr Ser Met Arg
              405   410   415

Pro Phe Thr Ala Thr Thr Lys Pro Glu His Glu Asp Gln Gly Leu Gln
              420   425   430

Ala Pro Ala Pro Pro His Ala Ala Ala Glu Pro Asp Leu Leu Tyr Tyr
              435   440   445

Pro Pro Gly Val Val Val Tyr Ser Gly Gly Arg Tyr Asp Leu Leu Pro
    450   455   460

Ser Ser Ser Ala Tyr
465

<210> 26
<211> 414
<212> PRT
<213> Mouse

<400> 26

Met Ala Thr Ser Asn Ser Ser Ala Ser Leu Pro Thr Leu Phe Trp Val
1   5   10   15

Asn Gly Ser Gly Asp Ser Val Leu Ser Thr Asp Gly Ala Ala Met Pro
              20   25   30

Val Gln Phe Leu Val Leu Arg Ile Met Val Ala Leu Ala Tyr Gly Leu
    35   40   45

Val Gly Ile Ile Gly Leu Leu Gly Asn Leu Ala Val Leu Trp Val Leu
50   55   60

Gly Asn Cys Gly Gln Arg Val Pro Gly Leu Ser Ser Asp Thr Phe Val
65   70   75   80

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Phe Ser Leu Ala Leu Ala Asp Leu Gly Leu Ala Leu Thr Leu Pro Phe
                85   90   95

Trp Ala Thr Glu Ser Ala Met Asp Phe His Trp Pro Phe Gly Ser Ala
            100   105   110

Leu Cys Lys Val Val Leu Thr Thr Thr Val Leu Ser Ile Tyr Ala Ser
        115   120   125

Thr Phe Leu Ile Thr Ala Leu Ser Ile Ala Arg Tyr Trp Val Val Ala
    130   135   140

Met Ala Val Gly Pro Gly Ser His Leu Ser Val Phe Trp Ala Arg Val
145   150   155   160

Val Thr Leu Ala Val Trp Val Ala Ala Ala Leu Val Thr Val Pro Thr
                165   170   175

Ala Ile Phe Gly Ala Glu Val Glu Leu Trp Gly Val Cys Leu Cys Leu
            180   185   190

Leu Arg Phe Pro Ser Arg Tyr Trp Leu Gly Ala Tyr Gln Leu Gln Arg
        195   200   205

Val Val Leu Ala Phe Ile Val Pro Leu Gly Val Ile Thr Thr Ser Tyr
    210   215   220

Leu Leu Leu Leu Ala Phe Leu Glu Arg Gln Gln Arg Cys Arg Pro Arg
225   230   235   240

Gln Trp Gln Asp Ser Arg Val Val Ala Arg Ser Val Arg Val Leu Val
            245   250   255

Ala Ser Phe Ala Leu Cys Trp Val Pro Asn His Val Val Thr Leu Trp
        260   265   270

Glu Ile Leu Val Arg Phe Asp Leu Val Pro Trp Asp Ser Thr Phe Tyr
        275   280   285

Thr Phe His Thr Tyr Ile Leu Pro Ile Thr Thr Cys Leu Ala His Ser
    290   295   300

Asn Ser Cys Leu Asn Pro Val Ile Tyr Cys Leu Leu Arg Arg Glu Pro
305   310   315   320

Gln Gln Val Leu Val Ser Ser Phe Arg Ala Leu Trp Ser Arg Leu Trp
            325   330   335

Pro Gln Arg Lys Ala Cys Met Glu Gln Met Ala Leu Lys Glu Val Gly
            340   345   350

Gly Arg Thr Val Ala Ser Thr Gln Glu Ser Gly Ser Ser Arg Thr His
                              Page 15

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                355                    360                    365

    Thr Asn Thr Met Glu His Leu Asp Glu Gly Cys Ser Leu Asn Thr Leu
        370                 375                 380

    Leu Ser Glu Thr Tyr Gln Gly Gln Ser Pro Gln Ile Leu Gly Arg Ser
    385                 390                 395                 400

    Ser Cys Ser Leu Ser Gln Ala Ala Val Ser Pro Gly Glu Val
                    405                 410
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<210>  27
<211>  374 .
<212>  PRT
<213>  Homo sapiens

<400>  27

Met Pro Thr Leu Asn Thr Ser Ala Ser Pro Pro Thr Phe Phe Trp Ala
1               5                  10                  15

Asn Ala Ser Gly Gly Ser Val Leu Ser Ala Asp Asp Ala Pro Met Pro
            20                  25                  30

Val Lys Phe Leu Ala Leu Arg Leu Met Val Ala Leu Ala Tyr Gly Leu
        35                  40                  45

Val Gly Ala Ile Gly Leu Leu Gly Asn Leu Ala Val Leu Trp Val Leu
    50                  55                  60

Ser Asn Cys Ala Arg Arg Ala Pro Gly Pro Pro Ser Asp Thr Phe Val
65                  70                  75                  80

Phe Asn Leu Ala Leu Ala Asp Leu Gly Leu Ala Leu Thr Leu Pro Phe
                85                  90                  95

Trp Ala Ala Glu Ser Ala Leu Asp Phe His Trp Pro Phe Gly Gly Ala
            100                 105                 110

Leu Cys Lys Met Val Leu Thr Ala Thr Val Leu Asn Val Tyr Ala Ser
        115                 120                 125

Ile Phe Leu Ile Thr Ala Leu Ser Val Ala Arg Tyr Trp Val Val Ala
    130                 135                 140

Met Ala Ala Gly Pro Gly Thr His Leu Ser Leu Phe Trp Ala Arg Ile
145                 150                 155                 160

Ala Thr Leu Ala Val Trp Ala Ala Ala Ala Leu Val Thr Val Pro Thr
                165                 170                 175

Ala Val Phe Gly Val Glu Gly Glu Val Cys Gly Val Arg Leu Cys Leu
                180                 185                 190
```

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```
Leu Arg Phe Pro Ser Arg Tyr Trp Leu Gly Ala Tyr Gln Leu Gln Arg
        195             200             205

Val Val Leu Ala Phe Met Val Pro Leu Gly Val Ile Thr Thr Ser Tyr
210             215             220

Leu Leu Leu Leu Ala Phe Leu Gln Arg Arg Gln Arg Arg Arg Gln Asp
225                 230             235             240

Ser Arg Val Val Ala Arg Ser Val Arg Ile Leu Val Ala Ser Phe Phe
            245             250             255

Leu Cys Trp Phe Pro Asn His Val Val Thr Leu Trp Gly Val Leu Val
        260             265             270

Lys Phe Asp Leu Val Pro Trp Asn Ser Thr Phe Tyr Thr Ile Gln Thr
        275             280             285

Tyr Val Phe Pro Val Thr Thr Cys Leu Ala His Ser Asn Ser Cys Leu
290             295             300

Asn Pro Val Leu Tyr Cys Leu Leu Arg Arg Glu Pro Arg Gln Ala Leu
305             310             315             320

Ala Gly Thr Phe Arg Asp Leu Arg Ser Arg Leu Trp Pro Gln Gly Gly
                325             330                 335

Gly Trp Val Gln Gln Val Ala Leu Lys Gln Val Gly Arg Arg Trp Val
            340             345             350

Ala Ser Asn Pro Arg Glu Ser Arg Pro Ser Thr Leu Leu Thr Asn Leu
        355             360             365

Asp Arg Gly Thr Pro Gly
    370
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<210>   28
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<213>   Human

<400>   28
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Arg Ala Ala Pro Tyr Gly Val Arg Leu Cys Gly Arg Glu Phe Ile Arg
1           5               10              15

Ala Val Ile Phe Thr Cys Gly Gly Ser Arg Trp Asp Val Leu Ala Gly
            20              25              30

Leu Ser Ser Ser Cys Cys Lys Trp Gly Cys Ser Lys Ser Glu Ile Ser
        35              40              45

Ser Leu Cys
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50

<210> 29
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<212> PRT
<213> Monkey

<400> 29

Met Pro Thr Leu Asn Thr Ser Ala Ser Pro Pro Thr Phe Trp Ala Asn
1   5   10   15

Ala Ser Gly Gly Ser Val Leu Ser Ala Asp Asp Ala Pro Met Pro Val
                20   25   30

Lys Phe Leu Ala Leu Arg Leu Met Val Ala Leu Ala Tyr Gly Leu Val
        35   40   45

Gly Ala Val Gly Leu Leu Gly Asn Leu Ala Val Leu Trp Val Leu Ser
    50   55   60

Asn Cys Ala Arg Arg Ala Pro Gly Pro Pro Ser Asp Thr Phe Val Phe
65   70   75   80

Asn Leu Ala Leu Ala Asp Leu Gly Leu Ala Leu Thr Leu Pro Phe Trp
                85   90   95

Ala Ala Glu Ser Ala Leu Asp Phe His Trp Pro Phe Gly Gly Ala Leu
                100   105   110

Cys Lys Met Val Leu Thr Ala Thr Val Leu Asn Val Tyr Ala Ser Ile
        115   120   125

Phe Leu Ile Thr Ala Leu Ser Val Ala Arg Tyr Trp Val Val Ala Met
    130   135   140

Ala Ala Gly Pro Gly Thr His Leu Ser Leu Phe Trp Ala Arg Ile Ala
145   150   155   160

Thr Leu Ala Val Trp Ala Ala Ala Ala Leu Val Thr Val Pro Thr Ala
                165   170   175

Val Phe Gly Val Glu Gly Glu Val Cys Gly Val Arg Leu Cys Leu Leu
                180   185   190

Arg Phe Pro Ser Arg Tyr Trp Leu Gly Ala Tyr Gln Leu Gln Arg Val
        195   200   205

Val Leu Ala Phe Met Val Pro Leu Gly Val Ile Thr Thr Ser Tyr Leu
    210   215   220

Leu Leu Leu Ala Phe Leu Gln Gln Arg Gln Arg Gln Gln Asp Ser
225   230   235   240

Arg Val Val Ala Arg Ser Val Arg Ile Leu Val Ala Ser Phe Phe Leu
                  245   250   255

Cys Trp Phe Pro Asn His Val Val Thr Leu Trp Gly Val Leu Val Lys
              260   265   270

Phe Asp Leu Val Pro Trp Asn Ser Thr Phe Tyr Thr Ile Gln Thr Tyr
          275   280   285

Val Leu Pro Val Thr Thr Cys Leu Ala His Ser Asn Ser Cys Leu Asn
      290   295   300

Pro Val Leu Tyr Cys Leu Leu Arg Arg Glu Pro Arg Gln Ala Leu Ala
305   310   315   320

Asp Thr Phe Arg Asp Leu Arg Ser Arg Leu Trp Pro Gln Gly Gly Gly
              325   330   335

Trp Val Gln Gln Val Ala Leu Lys Gln Val Gly Arg Arg Trp Val Ala
          340   345   350

Ser Asn Pro Pro Glu Ser Arg Pro Ser Thr Leu Leu Thr Asn Leu Asp
          355   360   365

Gly Gly Thr Pro Gly
          370

<210> 30
<211> 373
<212> PRT
<213> Cow

<400> 30

Met Pro Thr Pro Asn Thr Ser Ala Pro Leu Pro Ala Phe Trp Val Asn
1   5   10   15

Ala Ser Gly Gly Ser Val Leu Ser Ala Ala Asp Ala Thr Met Pro Val
          20   25   30

Gly Phe Leu Ala Leu Arg Val Ser Val Ala Leu Ala Tyr Gly Leu Val
          35   40   45

Gly Ala Val Gly Leu Leu Gly Asn Ser Ala Val Leu Trp Val Leu Gly
      50   55   60

Asn Cys Ala Gln Arg Ala Pro Cys Pro Pro Ser Asp Thr Phe Val Phe
65   70   75   80

Asn Leu Ala Leu Ala Asp Leu Gly Leu Ala Leu Thr Leu Pro Phe Trp
              85   90   95

Ala Ala Glu Ser Ala Leu Asp Phe His Trp Pro Phe Gly Gly Ala Leu
                            Page 19

                    100   105   110

Cys Lys Met Val Leu Thr Ala Thr Val Leu Asn Ile Tyr Ala Ser Ile
        115   120   125

Phe Leu Ile Thr Ala Leu Ser Val Ala Arg Tyr Trp Val Val Ala Met
    130   135   140

Ala Ala Gly Pro Gly Thr His Leu Ser Leu Phe Trp Ala Arg Val Ala
145   150   155   160

Thr Leu Ala Met Trp Val Ala Ala Ser Leu Val Thr Val Pro Thr Ala
            165   170   175

Val Phe Gly Ala Glu Gly Glu Val Ser Gly Val Arg Leu Cys Leu Leu
        180   185   190

Arg Phe Pro Ser Arg Tyr Trp Leu Gly Ala Tyr Gln Leu Gln Arg Val
        195   200   205

Val Leu Ala Phe Met Val Pro Leu Ser Ile Ile Thr Thr Ser Tyr Leu
    210   215   220

Leu Leu Leu Ala Phe Leu Arg Arg Arg Arg Arg Arg Trp Arg Asp Ser
225   230   235   240

Arg Gly Val Ala His Ser Ile Arg Ile Leu Leu Ala Ser Phe Phe Leu
            245   250   255

Cys Trp Phe Pro Asn His Val Val Thr Leu Trp Gly Val Leu Val Lys
            260   265   270

Phe Asp Leu Val Pro Trp Asp Ser Thr Phe Tyr Thr Val His Thr Tyr
        275   280   285

Val Phe Pro Val Thr Thr Cys Leu Ala His Thr Asn Ser Cys Leu Asn
    290   295   300

Pro Val Leu Tyr Cys Leu Leu Arg Gln Glu Pro Arg Gln Ala Leu Ala
305   310   315   320

Asp Thr Phe Arg Asp Leu Arg Ala Arg Leu Trp Pro Gln Gly Arg Gly
            325   330   335

Trp Val Glu Gln Val Ala Leu Lys Glu Met Gly Arg Arg Trp Thr Glu
        340   345   350

Ser Thr Pro Gln Glu Gly Gly Leu Ser Thr Met Leu Thr Asn Leu Asp
        355   360   365

Lys Gly Asn Pro Gly
    370

<210> 31
<211> 373
<212> PRT
<213> Pig

<400> 31

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Met Pro Thr Pro Asn Thr Ser Ala Pro Leu Pro Ala Phe Trp Val Asn
1               5                   10                  15

Ala Ser Gly Gly Ser Val Leu Ser Ala Asp Asp Ala Thr Met Pro Val
            20                  25                  30

Gly Phe Leu Ala Leu Arg Val Met Val Ala Leu Ala Tyr Gly Leu Val
        35                  40                  45

Gly Ala Val Gly Leu Leu Gly Asn Leu Ala Val Leu Trp Val Leu Gly
    50                  55                  60

Asn Cys Ala Arg Arg Ala Pro Cys Pro Pro Ser Asp Thr Phe Val Phe
65                  70                  75                  80

Asn Leu Ala Leu Ala Asp Leu Gly Leu Ala Leu Thr Leu Pro Phe Trp
                85                  90                  95

Ala Ala Glu Ser Ala Leu Asp Phe His Trp Pro Phe Gly Gly Ala Leu
            100                 105                 110

Cys Lys Met Ile Leu Thr Ala Thr Val Leu Asn Ile Tyr Ala Ser Ile
        115                 120                 125

Phe Leu Ile Thr Ala Leu Ser Val Ala Arg Tyr Trp Val Val Ala Met
    130                 135                 140

Ala Gly Gly Pro Gly Thr His Leu Ser Leu Phe Trp Ala Arg Val Ala
145                 150                 155                 160

Thr Leu Ala Val Trp Val Ala Ala Ala Leu Val Thr Val Pro Thr Ala
                165                 170                 175

Val Phe Gly Ala Glu Gly Glu Leu Cys Gly Val Arg Leu Cys Leu Leu
            180                 185                 190

Arg Phe Pro Ser Arg Tyr Trp Leu Gly Ala Tyr Gln Leu Gln Arg Val
        195                 200                 205

Val Leu Ala Phe Met Val Pro Leu Gly Ile Ile Thr Thr Ser Tyr Leu
    210                 215                 220

Leu Leu Leu Ala Phe Leu Arg Arg Arg Arg Gln Arg Arg Gln Asp Asn
225                 230                 235                 240
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Arg Val Val Ala Arg Ser Ile Arg Ile Leu Leu Ala Ser Phe Phe Leu
              245   250   255

Cys Trp Phe Pro Asn His Val Val Thr Phe Trp Gly Val Leu Val Lys
              260   265   270

Phe Asp Leu Val Pro Trp Asp Ser Thr Phe Tyr Thr Ile His Thr Tyr
              275   280   285

Val Phe Pro Val Thr Thr Cys Leu Ala His Ser Asn Ser Cys Leu Asn
    290   295   300

Pro Val Leu Tyr Cys Leu Leu Arg Arg Glu Pro Arg Arg Ala Leu Glu
305   310   315   320

Val Thr Phe Arg Asp Leu Arg Ala Arg Leu Trp Pro Gln Gly Arg Gly
              325   330   335

Trp Val Glu Gln Val Ala Leu Lys Glu Val Gly Arg Arg Trp Glu Glu
              340   345   350

Ile Thr Pro Arg Asp Gly Gly Pro Ser Ala Met Pro Thr Asn Arg Asp
          355   360   365

Lys Gly Thr Ala Gly
        370

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actagactgc aggccgccat gctgaccgca gcgt

<210>   33
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<212>   DNA
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tttctcaaac agtgccacgt agggtcgtcg tcggccccga agaaccccag g   111

<210>   34
<211>   111
<212>   DNA
<213>   Artificial

<220>
<223>   Human Relaxin-2 3' reverse PCR primer
                        Page 22

<400>   34
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tttattagcc aatgcactgt agagttgtcg tcggccccga agaaccccag g   111

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<211>   98
<212>   DNA
<213>   Artificial

<220>
<223>   Human INSL3 3' reverse primer

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gtagcgggcg gggtttcgtc ggccccgaag aaccccag   98

<210>   36
<211>   96
<212>   DNA
<213>   Artificial

<220>
<223>   Human INSL4 3' reverse primer

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gaaggggtcg aatcgtcggc cccgaagaac cccagg   96

<210>   37
<211>   108
<212>   DNA
<213>   Artificial

<220>
<223>   Human INSL5 3' reverse primer

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caaagtttgt aaatcttgtc gtcggccccg aagaacccca ggggttcc   108

<210>   38
<211>   98
<212>   DNA
<213>   Artificial

<220>
<223>   Human INSL6 3' reverse PCR primer

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cttctcggag tagcctcgtc ggccccgaag aaccccag   98

<210>   39
<211>   98
<212>   DNA
<213>   Artificial

<220>

<223> Human Insulin 3' reverse PCR primer

<400> 39
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ctgctccacg atgcctcgtc ggccccgaag aaccccag   98

<210> 40
<211> 98
<212> DNA
<213> Artificial

<220>
<223> Artificial A chain 3' reverse PCR primer

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gttctcgtac aggcctcgtc ggccccgaag aaccccag   98

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<212> DNA
<213> Artificial

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<223> Human LGR7 5' forward PCR primer

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<210> 42
<211> 41
<212> DNA
<213> Artificial

<220>
<223> Human LGR7 3' reverse PCR primer

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<210> 43
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<212> DNA
<213> Artificial

<220>
<223> Human LGR8 5' forward PCR primer

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<210> 44
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<212> DNA
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<211>   472
<212>   PRT
<213>   Mouse

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Met Gln Val Ala Ser Ala Thr Pro Ala Ala Thr Val Arg Lys Ala Ala
1   5   10   15

Ala Gly Asp Glu Leu Ser Glu Phe Phe Ala Leu Thr Pro Asp Leu Leu
            20   25   30

Glu Val Ala Asn Ala Ser Gly Asn Ala Ser Leu Gln Leu Gln Asp Leu
        35   40   45

Trp Trp Glu Leu Gly Leu Glu Leu Pro Asp Gly Ala Ala Pro Gly His
    50   55   60

Pro Pro Gly Gly Gly Gly Ala Glu Ser Thr Asp Thr Glu Ala Arg Val
65   70   75   80

Arg Ile Leu Ile Ser Ala Val Tyr Trp Val Val Cys Ala Leu Gly Leu
                85   90   95

Ala Gly Asn Leu Leu Val Leu Tyr Leu Met Lys Ser Lys Gln Gly Trp
            100   105   110

Arg Lys Ser Ser Ile Asn Leu Phe Val Thr Asn Leu Ala Leu Thr Asp
        115   120   125

Phe Gln Phe Val Leu Thr Leu Pro Phe Trp Ala Val Glu Asn Ala Leu
    130   135   140

Asp Phe Lys Trp Pro Phe Gly Lys Ala Met Cys Lys Ile Val Ser Met
145   150   155   160

Val Thr Ser Met Asn Met Tyr Ala Ser Val Phe Phe Leu Thr Ala Met
                165   170   175

Ser Val Ala Arg Tyr His Ser Val Ala Ser Ala Leu Lys Ser His Arg
            180   185   190

Thr Arg Gly Arg Gly Arg Gly Asp Cys Cys Gly Gln Ser Leu Arg Glu
        195   200   205

Ser Cys Cys Phe Ser Ala Lys Val Leu Cys Gly Leu Ile Trp Ala Ser
    210   215   220

Ala Ala Leu Ala Ser Leu Pro Asn Ala Ile Phe Ser Thr Thr Ile Arg
225   230   235   240

Val Leu Gly Glu Glu Leu Cys Leu Met His Phe Pro Asp Lys Leu Leu

                245   250   255

Gly Trp Asp Arg Gln Phe Trp Leu Gly Leu Tyr His Leu Gln Lys Val
            260   265   270

Leu Leu Gly Phe Leu Leu Pro Leu Ser Ile Ile Ser Leu Cys Tyr Leu
        275   280   285

Leu Leu Val Arg Phe Ile Ser Asp Arg Arg Val Val Gly Thr Thr Asp
    290   295   300

Ala Val Gly Ala Ala Ala Ala Pro Gly Gly Gly Leu Ser Thr Ala Ser
305   310   315   320

Ala Arg Arg Arg Ser Lys Val Thr Lys Ser Val Thr Ile Val Val Leu
                325   330   335

Ser Phe Phe Leu Cys Trp Leu Pro Asn Gln Ala Leu Thr Thr Trp Ser
            340   345   350

Ile Leu Ile Lys Phe Asn Ala Val Pro Phe Ser Gln Glu Tyr Phe Gln
        355   360   365

Cys Gln Val Tyr Ala Phe Pro Val Ser Val Cys Leu Ala His Ser Asn
    370   375   380

Ser Cys Leu Asn Pro Ile Leu Tyr Cys Leu Val Arg Arg Glu Phe Arg
385   390   395   400

Lys Ala Leu Lys Asn Leu Leu Trp Arg Ile Ala Ser Pro Ser Leu Thr
                405   410   415

Asn Met Arg Pro Phe Thr Ala Thr Thr Lys Pro Glu Pro Glu Asp His
            420   425   430

Gly Leu Gln Ala Leu Ala Pro Leu Asn Ala Ala Ala Glu Pro Asp Leu
        435   440   445

Ile Tyr Tyr Pro Pro Gly Val Val Val Tyr Ser Gly Gly Arg Tyr Asp
    450   455   460

Leu Leu Pro Ser Ser Ser Ala Tyr
465   470

<210>   46
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<212>   PRT
<213>   Rat

<400>   46
    Met Pro Lys Ala His Leu Ser Met Gln Val Ala Ser Ala Thr Thr Ala
    1   5   10   15

Ala Pro Met Ser Lys Ala Ala Ala Gly Asp Glu Leu Ser Gly Phe Phe
        20   25   30

Gly Leu Ile Pro Asp Leu Leu Glu Val Ala Asn Arg Ser Ser Asn Ala
        35   40   45

Ser Leu Gln Leu Gln Asp Leu Trp Trp Glu Leu Gly Leu Glu Leu Pro
    50   55   60

Asp Gly Ala Ala Pro Gly His Pro Pro Gly Ser Gly Gly Ala Glu Ser
65   70   75   80

Ala Asp Thr Glu Ala Arg Val Arg Ile Leu Ile Ser Ala Val Tyr Trp
            85   90   95

Val Val Cys Ala Leu Gly Leu Ala Gly Asn Leu Leu Val Leu Tyr Leu
        100   105   110

Met Lys Ser Lys Gln Gly Trp Arg Lys Ser Ser Ile Asn Leu Phe Val
    115   120   125

Thr Asn Leu Ala Leu Thr Asp Phe Gln Phe Val Leu Thr Leu Pro Phe
    130   135   140

Trp Ala Val Glu Asn Ala Leu Asp Phe Lys Trp Pro Phe Gly Lys Ala
145   150   155   160

Met Cys Lys Ile Val Ser Met Val Thr Ser Met Asn Met Tyr Ala Ser
            165   170   175

Val Phe Phe Leu Thr Ala Met Ser Val Ala Arg Tyr His Ser Val Ala
        180   185   190

Ser Ala Leu Lys Ser His Arg Thr Arg Gly His Gly Arg Gly Asp Cys
    195   200   205

Cys Gly Gln Ser Leu Gly Glu Ser Cys Cys Phe Ser Ala Lys Val Leu
    210   215   220

Cys Gly Leu Ile Trp Ala Ser Ala Ala Ile Ala Ser Leu Pro Asn Val
225   230   235   240

Ile Phe Ser Thr Thr Ile Asn Val Leu Gly Glu Glu Leu Cys Leu Met
            245   250   255

His Phe Pro Asp Lys Leu Leu Gly Trp Asp Arg Gln Phe Trp Leu Gly
            260   265   270

Leu Tyr His Leu Gln Lys Val Leu Leu Gly Phe Leu Leu Pro Leu Ser
    275   280   285

Ile Ile Ser Leu Cys Tyr Leu Leu Leu Val Arg Phe Ile Ser Asp Arg
290   295   300

Arg Val Val Gly Thr Thr Asp Gly Ala Thr Ala Pro Gly Gly Ser Leu
305   310   315   320

Ser Thr Ala Gly Ala Arg Arg Arg Ser Lys Val Thr Lys Ser Val Thr
              325   330   335

Ile Val Val Leu Ser Phe Phe Leu Cys Trp Leu Pro Asn Gln Ala Leu
              340   345   350

Thr Thr Trp Ser Ile Leu Ile Lys Phe Asn Val Val Pro Phe Ser Gln
         355   360   365

Glu Tyr Phe Gln Cys Gln Val Tyr Ala Phe Pro Val Ser Val Cys Leu
    370   375   380

Ala His Ser Asn Ser Cys Leu Asn Pro Ile Leu Tyr Cys Leu Val Arg
385   390   395   400

Arg Glu Phe Arg Lys Ala Leu Lys Asn Leu Leu Trp Arg Ile Ala Ser
              405   410   415

Pro Ser Leu Thr Ser Met Arg Pro Phe Thr Ala Thr Thr Lys Pro Glu
         420   425   430

Pro Glu Asp His Gly Leu Gln Ala Leu Ala Pro Leu Asn Ala Thr Ala
         435   440   445

Glu Pro Asp Leu Ile Tyr Tyr Pro Pro Gly Val Val Val Tyr Ser Gly
    450   455   460

Gly Arg Tyr Asp Leu Leu Pro Ser Ser Ser Ala Tyr
465   470   475

<210>   47
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<212>   PRT
<213>   Rat

<400>   47

Met Gln Val Ala Ser Ala Thr Thr Ala Ala Pro Met Ser Lys Ala Ala
1   5   10   15

Ala Gly Asp Glu Leu Ser Gly Phe Phe Gly Leu Ile Pro Asp Leu Leu
              20   25   30

Glu Val Ala Asn Arg Ser Ser Asn Ala Ser Leu Gln Leu Gln Asp Leu
         35   40   45

Trp Trp Glu Leu Gly Leu Glu Leu Pro Asp Gly Ala Ala Pro Gly His
50   55   60

Pro Pro Gly Ser Gly Gly Ala Glu Ser Ala Asp Thr Glu Ala Arg Val
65   70   75   80

Arg Ile Leu Ile Ser Ala Val Tyr Trp Val Val Cys Ala Leu Gly Leu
                85   90   95

Ala Gly Asn Leu Leu Val Leu Tyr Leu Met Lys Ser Lys Gln Gly Trp
            100   105   110

Arg Lys Ser Ser Ile Asn Leu Phe Val Thr Asn Leu Ala Leu Thr Asp
        115   120   125

Phe Gln Phe Val Leu Thr Leu Pro Phe Trp Ala Val Glu Asn Ala Leu
    130   135   140

Asp Phe Lys Trp Pro Phe Gly Lys Ala Met Cys Lys Ile Val Ser Met
145   150   155   160

Val Thr Ser Met Asn Met Tyr Ala Ser Val Phe Phe Leu Thr Ala Met
            165   170   175

Ser Val Ala Arg Tyr His Ser Val Ala Ser Ala Leu Lys Ser His Arg
            180   185   190

Thr Arg Gly His Gly Arg Gly Asp Cys Cys Gly Gln Ser Leu Gly Glu
        195   200   205

Ser Cys Cys Phe Ser Ala Lys Val Leu Cys Gly Leu Ile Trp Ala Ser
    210   215   220

Ala Ala Ile Ala Ser Leu Pro Asn Val Ile Phe Ser Thr Thr Ile Asn
225   230   235   240

Val Leu Gly Glu Glu Leu Cys Leu Met His Phe Pro Asp Lys Leu Leu
            245   250   255

Gly Trp Asp Arg Gln Phe Trp Leu Gly Leu Tyr His Leu Gln Lys Val
            260   265   270

Leu Leu Gly Phe Leu Leu Pro Leu Ser Ile Ile Ser Leu Cys Tyr Leu
    275   280   285

Leu Leu Val Arg Phe Ile Ser Asp Arg Arg Val Val Gly Thr Thr Asp
    290   295   300

Gly Ala Thr Ala Pro Gly Gly Ser Leu Ser Thr Ala Gly Ala Arg Arg
305   310   315   320

Arg Ser Lys Val Thr Lys Ser Val Thr Ile Val Val Leu Ser Phe Phe
            325   330   335

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Leu Cys Trp Leu Pro Asn Gln Ala Leu Thr Thr Trp Ser Ile Leu Ile
        340   345   350

Lys Phe Asn Val Val Pro Phe Ser Gln Glu Tyr Phe Gln Cys Gln Val
        355   360   365

Tyr Ala Phe Pro Val Ser Val Cys Leu Ala His Ser Asn Ser Cys Leu
        370   375   380

Asn Pro Ile Leu Tyr Cys Leu Val Arg Arg Glu Phe Arg Lys Ala Leu
385   390   395   400

Lys Asn Leu Leu Trp Arg Ile Ala Ser Pro Ser Leu Thr Ser Met Arg
                    405   410   415

Pro Phe Thr Ala Thr Thr Lys Pro Glu Pro Glu Asp His Gly Leu Gln
                420   425   430

Ala Leu Ala Pro Leu Asn Ala Thr Ala Glu Pro Asp Leu Ile Tyr Tyr
        435   440   445

Pro Pro Gly Val Val Val Tyr Ser Gly Gly Arg Tyr Asp Leu Leu Pro
450   455   460

Ser Ser Ser Ala Tyr
465

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<212> DNA
<213> Mouse

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gcggtcatct tcacttgcgg aggctcacga tggcgccggg cggacatctt ggcccacgaa   180

tctctggggg acttcttcgc tgatggagaa gccaatacag accacctggc cagcgagctg   240

gatgaagcgg tgggctccag cgagtggctg gccctaacca aatcccccca ggctttctac   300

ggtggtcgag ccagctggca aggtcacct ggagtggttc ggggcagcag agatgtgttg   360

gctggccttt ccagcagttg ctgcgagtgg ggctgtagca gagccaaat tagcagcttg   420
                                                                     426
tgctag

<210> 49
<211> 141
<212> PRT
<213> Mouse

<400> 49

Met Ala Met Leu Gly Leu Leu Leu Leu Ala Ser Trp Ala Leu Leu Gly
1   5   10   15
Page 30

Ala Leu Gly Leu Gln Ala Glu Ala Arg Pro Ala Pro Tyr Gly Val Lys
          20   25   30

Leu Cys Gly Arg Glu Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly
          35   40   45

Ser Arg Trp Arg Arg Ala Asp Ile Leu Ala His Glu Ser Leu Gly Asp
          50   55   60

Phe Phe Ala Asp Gly Glu Ala Asn Thr Asp His Leu Ala Ser Glu Leu
65   70   75   80

Asp Glu Ala Val Gly Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro
                  85   90   95

Gln Ala Phe Tyr Gly Gly Arg Ala Ser Trp Gln Gly Ser Pro Gly Val
              100   105   110

Val Arg Gly Ser Arg Asp Val Leu Ala Gly Leu Ser Ser Ser Cys Cys
          115   120   125

Glu Trp Gly Cys Ser Lys Ser Gln Ile Ser Ser Leu Cys
          130   135   140

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gtcatcttca cctgcggggg ctcacgatgg cgccgggcgg acatcttggc ccacgaccct   180

ctgggggaat tcttcgctga tggagaagcc aatacagacc acctggccag cgaactggac   240

gaagctgtgg gctccagcga gtggctggcc ctgaccaaat cccccccaggt cttctatggg   300

ggtcgatcca gctggcaagg gtctcccgga gtggttcggg gcagcagaga tgtgctggct   360

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tag   423

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<212>   PRT
<213>   Rat

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1   5   10   15

Leu Val Leu Gln Ala Glu Ala Arg Pro Ala Pro Tyr Gly Val Lys Leu

                    20   25   30

Cys Gly Arg Glu Phe Ile Arg Ala Val Ile Phe Thr Cys Gly Gly Ser
        35   40   45

Arg Trp Arg Arg Ala Asp Ile Leu Ala His Asp Pro Leu Gly Glu Phe
    50   55   60

Phe Ala Asp Gly Glu Ala Asn Thr Asp His Leu Ala Ser Glu Leu Asp
65   70   75   80

Glu Ala Val Gly Ser Ser Glu Trp Leu Ala Leu Thr Lys Ser Pro Gln
                85   90   95

Val Phe Tyr Gly Gly Arg Ser Ser Trp Gln Gly Ser Pro Gly Val Val
            100   105   110

Arg Gly Ser Arg Asp Val Leu Ala Gly Leu Ser Ser Ser Cys Cys Glu
        115   120   125

Trp Gly Cys Ser Lys Ser Gln Ile Ser Ser Leu Cys
    130   135   140

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<212>   DNA
<213>   Human

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gccctatctg agaggcaacc atcattacca gagctacagc agtatgtacc tgcattaaag   360

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<210>   53
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<212>   PRT
<213>   Human

<400>   53
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Met Pro Arg Leu Phe Leu Phe His Leu Leu Glu Phe Cys Leu Leu Leu
1   5   10   15

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Asn Gln Phe Ser Arg Ala Val Ala Ala Lys Trp Lys Asp Asp Val Ile
            20              25              30

Lys Leu Cys Gly Arg Glu Leu Val Arg Ala Gln Ile Ala Ile Cys Gly
        35              40              45

Met Ser Thr Trp Ser Lys Arg Ser Leu Ser Gln Glu Asp Ala Pro Gln
        50              55              60

Thr Pro Arg Pro Val Ala Glu Ile Val Pro Ser Phe Ile Asn Lys Asp
65              70              75              80

Thr Glu Thr Ile Ile Ile Met Leu Glu Phe Ile Ala Asn Leu Pro Pro
            85              90              95

Glu Leu Lys Ala Ala Leu Ser Glu Arg Gln Pro Ser Leu Pro Glu Leu
            100             105             110

Gln Gln Tyr Val Pro Ala Leu Lys Asp Ser Asn Leu Ser Phe Glu Glu
        115             120             125

Phe Lys Lys Leu Ile Arg Asn Arg Gln Ser Glu Ala Ala Asp Ser Asn
    130             135             140

Pro Ser Glu Leu Lys Tyr Leu Gly Leu Asp Thr His Ser Gln Lys Lys
145             150             155             160

Arg Arg Pro Tyr Val Ala Leu Phe Glu Lys Cys Cys Leu Ile Gly Cys
                165             170             175

Thr Lys Arg Ser Leu Ala Lys Tyr Cys
            180             185
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<212>   DNA
<213>   Human
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cgcgcgcaga ttgccatttg cggcatgagc acctggagca aaaggtctct gagccaggaa    180
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acagaaacca taaatatgat gtcagaattt gttgctaatt gccacaggag ctgaagttga    300
accctgtctg agatgcagcc agcattacca cagctacaac aacatgtacc tgtattaaaa    360
gattccagtc ttctctttga agaatttaag aaacttattc gcaatagaca aagtgaagcc    420
gcagacagca gtccttcaga attaaaatac ttaggcttgg atactcattc tcgaaaaaag    480
agacaactct acagtgcatt ggctaataaa tgttgccatg ttggttgtac caaaagatct    540
cttgctagat tttgctga                                                   558
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<210> 55
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<212> PRT
<213> Human

<400> 55

Met Pro Arg Leu Phe Phe Phe His Leu Leu Gly Val Cys Leu Leu Leu
1   5   10   15

Asn Gln Phe Ser Arg Ala Val Ala Asp Ser Trp Met Glu Glu Val Ile
                20   25   30

Lys Leu Cys Gly Arg Glu Leu Val Arg Ala Gln Ile Ala Ile Cys Gly
            35   40   45

Met Ser Thr Trp Ser Lys Arg Ser Leu Ser Gln Glu Asp Ala Pro Gln
        50   55   60

Thr Pro Arg Pro Val Ala Glu Ile Val Pro Ser Phe Ile Asn Lys Asp
65   70   75   80

Thr Glu Thr Ile Asn Met Met Ser Glu Phe Val Ala Asn Leu Pro Gln
                85   90   95

Glu Leu Lys Leu Thr Leu Ser Glu Met Gln Pro Ala Leu Pro Gln Leu
            100   105   110

Gln Gln His Val Pro Val Leu Lys Asp Ser Ser Leu Leu Phe Glu Glu
        115   120   125

Phe Lys Lys Leu Ile Arg Asn Arg Gln Ser Glu Ala Ala Asp Ser Ser
    130   135   140

Pro Ser Glu Leu Lys Tyr Leu Gly Leu Asp Thr His Ser Arg Lys Lys
145   150   155   160

Arg Gln Leu Tyr Ser Ala Leu Ala Asn Lys Cys Cys His Val Gly Cys
                165   170   175

Thr Lys Arg Ser Leu Ala Arg Phe Cys
            180   185

<210> 56
<211> 549
<212> DNA
<213> Pig

<400> 56
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ctgtgggtgg agatctgtgg ctccgtctcc tggggaagaa ctgctctcag cctggaagag   180

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cctcagctgg aaactggacc cccggcagaa accatgccat cctccatcac caaagatgca   240

gaaatcttaa agatgatgtt ggaatttgtt cctaatttgc cacaggagct gaaggcaaca   300

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cttaactttg aagaatttaa gaaaattatt cttaacagac aaaatgaagc agaagacaaa   420

agtcttttag aattaaaaaa cttaggtttta gataaacatt ccagaaaaaa gagactgttc   480

cgtatgacac tgagcgagaa atgttgtcaa gtaggttgta tcagaaaaga tattgctaga   540

ttatgctga   549

<210>   57
<211>   182
<212>   PRT
<213>   Pig

<400>   57

Met Pro Arg Leu Phe Ser Tyr Leu Leu Gly Val Trp Leu Leu Leu Ser
1   5   10   15

Gln Leu Pro Arg Glu Ile Pro Gly Gln Ser Thr Asn Asp Phe Ile Lys
                20   25   30

Ala Cys Gly Arg Glu Leu Val Arg Leu Trp Val Glu Ile Cys Gly Ser
            35   40   45

Val Ser Trp Gly Arg Thr Ala Leu Ser Leu Glu Glu Pro Gln Leu Glu
        50   55   60

Thr Gly Pro Pro Ala Glu Thr Met Pro Ser Ser Ile Thr Lys Asp Ala
65   70   75   80

Glu Ile Leu Lys Met Met Leu Glu Phe Val Pro Asn Leu Pro Gln Glu
                85   90   95

Leu Lys Ala Thr Leu Ser Glu Arg Gln Pro Ser Leu Arg Glu Leu Gln
                100   105   110

Gln Ser Ala Ser Lys Asp Ser Asn Leu Asn Phe Glu Glu Phe Lys Lys
            115   120   125

Ile Ile Leu Asn Arg Gln Asn Glu Ala Glu Asp Lys Ser Leu Leu Glu
    130   135   140

Leu Lys Asn Leu Gly Leu Asp Lys His Ser Arg Lys Lys Arg Leu Phe
145   150   155   160

Arg Met Thr Leu Ser Glu Lys Cys Cys Gln Val Gly Cys Ile Arg Lys
                165   170   175

Asp Ile Ala Arg Leu Cys
                180

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<210>   58
<211>   558
<212>   DNA
<213>   Mouse

<400>   58
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gcccgtgaat tgatcaaaat ctgcgggggcc tccgtgggaa gattggcttt gagccaggag    180

gagccagctc tgcttgccag gcaagccact gaagttgtgc catccttcat caacaaagat     240

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gtactgtctg aggctcaggc ctcgctccca gagctacaac acgcacctgt gttgagcgat     360

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gagtctggtg gattgatgag ccagcaatgt tgccacgtcg gttgtagcag aagatctatt     540
                                                                       558
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<210>   59
<211>   185
<212>   PRT
<213>   Mouse

<400>   59
Met Ser Ser Arg Phe Leu Leu Gln Leu Leu Gly Phe Trp Leu Leu Leu
1               5                   10                  15

Ser Gln Pro Cys Arg Thr Arg Val Ser Glu Glu Trp Met Asp Gly Phe
            20                  25                  30

Ile Arg Met Cys Gly Arg Glu Tyr Ala Arg Glu Leu Ile Lys Ile Cys
        35                  40                  45

Gly Ala Ser Val Gly Arg Leu Ala Leu Ser Gln Glu Glu Pro Ala Leu
    50                  55                  60

Leu Ala Arg Gln Ala Thr Glu Val Val Pro Ser Phe Ile Asn Lys Asp
65                  70                  75                  80

Ala Glu Pro Phe Asp Thr Thr Leu Lys Cys Leu Pro Asn Leu Ser Glu
                85                  90                  95

Glu Leu Lys Ala Val Leu Ser Glu Ala Gln Ala Ser Leu Pro Glu Leu
            100                 105                 110

Gln His Ala Pro Val Leu Ser Asp Ser Val Val Ser Leu Glu Gly Phe
        115                 120                 125

Lys Lys Thr Leu His Asp Lys Leu Gly Glu Ala Glu Asp Gly Ser Pro
    130                 135                 140
```

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```
Pro Gly Leu Lys Tyr Leu Gln Ser Asp Thr His Ser Arg Lys Lys Arg
145             150             155             160
```

```
Glu Ser Gly Gly Leu Met Ser Gln Gln Cys Cys His Val Gly Cys Ser
                165             170             175
```

```
Arg Arg Ser Ile Ala Lys Leu Tyr Cys
            180             185
```

```
<210>   60
<211>   561
<212>   DNA
<213>   Rat
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agggcgcgag tctcggagga gtggatggac caagtcattc aggtgtgcgg ccgtggatat     120
gcccgcgcat ggatcgaagt ctgcgggggcc tccgtgggaa gactggcttt gagccaggag     180
gagccagctc cgctagccag caagccact gcagaagttg tgccatcctt catcaacaaa     240
gatgcggagc ctttcgatat gacgttgaaa tgccttccaa atttgtctga ggagcggaag     300
gcagcactgt ctgaggggcg agcaccgttc ccagagctac aacaacacgc acccgcgttg     360
agcgattccg ttgttagctt ggaaggattt aagaaaactt tccacaatca gctgggtgaa     420
gcagaagatg gcggtcctcc agagctcaaa tacttaggct cagatgctca gtcacggaaa     480
aagaggcagt ctggcgcact gctcagtgag cagtgttgcc acatcggttg taccagaaga     540
tccattgcta aactctgctg a                                               561
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<210>   61
<211>   186
<212>   PRT
<213>   Rat
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<400>   61
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Met Ser Ser Arg Leu Leu Leu Gln Leu Leu Gly Phe Trp Leu Phe Leu
1               5               10              15
```

```
Ser Gln Pro Cys Arg Ala Arg Val Ser Glu Glu Trp Met Asp Gln Val
            20              25              30
```

```
Ile Gln Val Cys Gly Arg Gly Tyr Ala Arg Ala Trp Ile Glu Val Cys
                35              40              45
```

```
Gly Ala Ser Val Gly Arg Leu Ala Leu Ser Gln Glu Glu Pro Ala Pro
    50              55              60
```

```
Leu Ala Arg Gln Ala Thr Ala Glu Val Val Pro Ser Phe Ile Asn Lys
65              70              75              80
```

```
Asp Ala Glu Pro Phe Asp Met Thr Leu Lys Cys Leu Pro Asn Leu Ser
```

                85   90   95

Glu Glu Arg Lys Ala Ala Leu Ser Glu Gly Arg Ala Pro Phe Pro Glu
            100   105   110

Leu Gln Gln His Ala Pro Ala Leu Ser Asp Ser Val Val Ser Leu Glu
            115   120   125

Gly Phe Lys Lys Thr Phe His Asn Gln Leu Gly Glu Ala Glu Asp Gly
    130   135   140

Gly Pro Pro Glu Leu Lys Tyr Leu Gly Ser Asp Ala Gln Ser Arg Lys
145   150   155   160

Lys Arg Gln Ser Gly Ala Leu Leu Ser Glu Gln Cys Cys His Ile Gly
                165   170   175

Cys Thr Arg Arg Ser Ile Ala Lys Leu Cys
            180   185

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<212> DNA
<213> Human

<400> 62
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gcgctagtgc gcgtgtgcgg gggcccccgc tggtccaccg aagccaggag gcctgcggcc   180

ggaggcgacc gtgagttgct acagtggctg gagagacgac atctgctcca tgggctggtg   240

gccgacagta atctcacgct gggacctggc ctgcagcccc tgccccagac ctctcaccat   300

caccgccacc accgtgcagc tgccaccaac cctgcacgct actgctgcct cagtggctgt   360

acccaacaag acctgctgac cctctgtccc tactga   396

<210> 63
<211> 131
<212> PRT
<213> Human

<400> 63

Met Asp Pro Arg Leu Pro Ala Trp Ala Leu Val Leu Leu Gly Pro Ala
1   5   10   15

Leu Val Phe Ala Leu Gly Pro Ala Pro Thr Pro Glu Met Arg Glu Lys
                20   25   30

Leu Cys Gly His His Phe Val Arg Ala Leu Val Arg Val Cys Gly Gly
        35   40   45

Pro Arg Trp Ser Thr Glu Ala Arg Arg Pro Ala Ala Gly Gly Asp Arg
    50   55   60

Glu Leu Leu Gln Trp Leu Glu Arg Arg His Leu Leu His Gly Leu Val
65   70   75   80

Ala Asp Ser Asn Leu Thr Leu Gly Pro Gly Leu Gln Pro Leu Pro Gln
                85   90   95

Thr Ser His His His Arg His His Arg Ala Ala Ala Thr Asn Pro Ala
                100   105   110

Arg Tyr Cys Cys Leu Ser Gly Cys Thr Gln Gln Asp Leu Leu Thr Leu
        115   120   125

Cys Pro Tyr
        130

<210> 64
<211> 369
<212> DNA
<213> Mouse

<400> 64
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tgcggcggcc cacgctggtc gcccgaggcc acgcagcctg tggagacccg ggaccgggag   180

ctgctgcagt ggctagagca gagacatctc ctgcacgcgc tggtggccga cgtggaccca   240

gcgctagacc cgcagcttcc tcggcaggct tctcagcgcc agcgccgcag tgccgccacc   300

aacgctgtgc accgctgctg tcttactggc tgcacccagc aagacctgtt gggtctgtgt   360

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<210> 65
<211> 122
<212> PRT
<213> Mouse

<400> 65

Met Arg Ala Pro Leu Leu Leu Met Leu Leu Ala Leu Gly Ser Ala Leu
1   5   10   15

Arg Ser Pro Gln Pro Pro Glu Ala Arg Ala Lys Leu Cys Gly His His
                20   25   30

Leu Val Arg Thr Leu Val Arg Val Cys Gly Gly Pro Arg Trp Ser Pro
        35   40   45

Glu Ala Thr Gln Pro Val Glu Thr Arg Asp Arg Glu Leu Leu Gln Trp
        50   55   60

Leu Glu Gln Arg His Leu Leu His Ala Leu Val Ala Asp Val Asp Pro
65   70   75   80

Ala Leu Asp Pro Gln Leu Pro Arg Gln Ala Ser Gln Arg Gln Arg Arg
                 85   90   95

Ser Ala Ala Thr Asn Ala Val His Arg Cys Cys Leu Thr Gly Cys Thr
             100   105   110

Gln Gln Asp Leu Leu Gly Leu Cys Pro His
             115   120

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<212> DNA
<213> Rat

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tgcggtggcc cgcgttggtc acccgaggcc acgcagcctg tggacacccg cgaccgggag   180

ctgctgcagt ggctggagca acgacatctc ctgcacgcgc tcgtggccga tgcggatccc   240

gcgctagacc cggaccccgc gctggacccg cagcttcctc accaggcttc tcagcgccag   300

cgccgcagtg tggccaccaa cgctgtgcac cgctgctgtc tcactggctg cacccagcaa   360

gaccttttgg gtctgtgtcc ccactga   387

<210> 67
<211> 128
<212> PRT
<213> Rat

<400> 67

Met His Ala Leu Leu Leu Leu Leu Leu Ala Leu Gly Ser Ala Leu
1   5   10   15

Arg Ser Pro Gln Pro Pro Glu Ala Arg Ala Lys Leu Cys Gly His His
             20   25   30

Leu Val Arg Ala Leu Val Arg Val Cys Gly Gly Pro Arg Trp Ser Pro
             35   40   45

Glu Ala Thr Gln Pro Val Asp Thr Arg Asp Arg Glu Leu Leu Gln Trp
     50   55   60

Leu Glu Gln Arg His Leu Leu His Ala Leu Val Ala Asp Ala Asp Pro
65   70   75   80

Ala Leu Asp Pro Asp Pro Ala Leu Asp Pro Gln Leu Pro His Gln Ala
                 85   90   95

Ser Gln Arg Gln Arg Arg Ser Val Ala Thr Asn Ala Val His Arg Cys
             100   105   110

Cys Leu Thr Gly Cys Thr Gln Gln Asp Leu Leu Gly Leu Cys Pro His
        115   120   125

<210>   68
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<212>   DNA
<213>   Human

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acgacatcag aattcattcc taatttgtca ccagagctga agaaaccact gtctgaaggg   300

cagccatcat tgaagaaaat aatactttcc cgcaaaaaga gaagtggacg tcacagattt   360

gatccattct gttgtgaagt aaatttgtgac gatggaactt cagttaaatt atgtacatag   420

<210>   69
<211>   139
<212>   PRT
<213>   Human

<400>   69

Met Ala Ser Leu Phe Arg Ser Tyr Leu Pro Ala Ile Trp Leu Leu Leu
1   5   10   15

Ser Gln Leu Leu Arg Glu Ser Leu Ala Ala Glu Leu Arg Gly Cys Gly
            20   25   30

Pro Arg Phe Gly Lys His Leu Leu Ser Tyr Cys Pro Met Pro Glu Lys
        35   40   45

Thr Phe Thr Thr Thr Pro Gly Gly Trp Leu Leu Glu Ser Gly Arg Pro
    50   55   60

Lys Glu Met Val Ser Thr Ser Asn Asn Lys Asp Gly Gln Ala Leu Gly
65   70   75   80

Thr Thr Ser Glu Phe Ile Pro Asn Leu Ser Pro Glu Leu Lys Lys Pro
                85   90   95

Leu Ser Glu Gly Gln Pro Ser Leu Lys Lys Ile Ile Leu Ser Arg Lys
            100   105   110

Lys Arg Ser Gly Arg His Arg Phe Asp Pro Phe Cys Cys Glu Val Ile
        115   120   125

Cys Asp Asp Gly Thr Ser Val Lys Leu Cys Thr
    130   135

<210>   70

<211>   408
<212>   DNA
<213>   Human

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tgtgctagct ccaggtggag aaggcatctg gaggggatcc ctcaagctca gcaagctgag   180

acaggaaact ccttccagct cccacataaa cgtgagtttt ctgaggaaaa tccagcgcaa   240

aaccttccga aggtggatgc ctcaggggaa gaccgtcttt ggggtggaca gatgcccact   300

gaagagcttt ggaagtcaaa gaagcattca gtgatgtcaa gacaagattt acaaactttg   360

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<210>   71
<211>   135
<212>   PRT
<213>   Human

<400>   71

Met Lys Gly Ser Ile Phe Thr Leu Phe Leu Phe Ser Val Leu Phe Ala
1   5   10   15

Ile Ser Glu Val Arg Ser Lys Glu Ser Val Arg Leu Cys Gly Leu Glu
                20   25   30

Tyr Ile Arg Thr Val Ile Tyr Ile Cys Ala Ser Ser Arg Trp Arg Arg
            35   40   45

His Leu Glu Gly Ile Pro Gln Ala Gln Gln Ala Glu Thr Gly Asn Ser
        50   55   60

Phe Gln Leu Pro His Lys Arg Glu Phe Ser Glu Glu Asn Pro Ala Gln
65   70   75   80

Asn Leu Pro Lys Val Asp Ala Ser Gly Glu Asp Arg Leu Trp Gly Gly
                85   90   95

Gln Met Pro Thr Glu Glu Leu Trp Lys Ser Lys Lys His Ser Val Met
                100   105   110

Ser Arg Gln Asp Leu Gln Thr Leu Cys Cys Thr Asp Gly Cys Ser Met
            115   120   125

Thr Asp Leu Ser Ala Leu Cys
        130   135

<210>   72
<211>   408
<212>   DNA
<213>   Mouse

<400>   72

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gaaggtcttt gggaactgaa gaagcactca gtggtatcca gacgagatct gcaagctctg   360

tgctgcaggg aaggctgctc catgaaggaa ctcagcaccc tctgttag              408
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<210>   73
<211>   135
<212>   PRT
<213>   Mouse

<400>   73

Met Lys Gly Pro Thr Leu Ala Leu Phe Leu Leu Leu Val Leu Leu Ala
1               5                   10                  15


Val Val Glu Val Arg Ser Arg Gln Thr Val Lys Leu Cys Gly Leu Asp
            20                  25                  30


Tyr Val Arg Thr Val Ile Tyr Ile Cys Ala Ser Ser Arg Trp Arg Arg
        35                  40                  45


His Leu Glu Gly His Phe His Ser Gln Gln Ala Glu Thr Arg Asn Tyr
    50                  55                  60


Leu Gln Leu Leu Asp Arg His Glu Pro Ser Lys Lys Thr Leu Glu His
65                  70                  75                  80


Ser Leu Pro Lys Thr Asp Leu Ser Gly Gln Glu Leu Val Arg Asp Pro
                85                  90                  95


Gln Ala Pro Lys Glu Gly Leu Trp Glu Leu Lys Lys His Ser Val Val
            100                 105                 110


Ser Arg Arg Asp Leu Gln Ala Leu Cys Cys Arg Glu Gly Cys Ser Met
        115                 120                 125


Lys Glu Leu Ser Thr Leu Cys
    130                 135
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<210>   74
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<212>   DNA
<213>   Human

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atagaaaaac tctgcggcca tgccaactgg agccagttcc gtttcgagga ggaaacccct   180
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acaggatgta caaaagaaga acttagcatt gcatgtcttc catatattga ttttaaaagg   600

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<210> 75
<211> 213
<212> PRT
<213> Human

<400> 75

Met Pro Arg Leu Leu Arg Leu Ser Leu Leu Trp Leu Gly Leu Leu Leu
1   5   10   15

Val Arg Phe Ser Arg Glu Leu Ser Asp Ile Ser Ser Ala Arg Lys Leu
            20   25   30

Cys Gly Arg Tyr Leu Val Lys Glu Ile Glu Lys Leu Cys Gly His Ala
        35   40   45

Asn Trp Ser Gln Phe Arg Phe Glu Glu Glu Thr Pro Phe Ser Arg Leu
    50   55   60

Ile Ala Gln Ala Ser Glu Lys Val Glu Ala Tyr Ser Pro Tyr Gln Phe
65   70   75   80

Glu Ser Pro Gln Thr Ala Ser Pro Ala Arg Gly Arg Gly Thr Asn Pro
                85   90   95

Val Ser Thr Ser Trp Glu Glu Ala Val Asn Ser Trp Glu Met Gln Ser
            100   105   110

Leu Pro Glu Tyr Lys Asp Lys Lys Gly Tyr Ser Pro Leu Gly Lys Thr
        115   120   125

Arg Glu Phe Ser Ser Ser His Asn Ile Asn Val Tyr Ile His Glu Asn
    130   135   140

Ala Phe Phe Gln Lys Lys Arg Arg Asn Lys Ile Lys Thr Leu Ser Asn
145   150   155   160

Leu Phe Trp Gly His His Pro Gln Arg Lys Arg Arg Gly Tyr Ser Glu
                165   170   175

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Lys Cys Cys Leu Thr Gly Cys Thr Lys Glu Glu Leu Ser Ile Ala Cys
            180   185   190

Leu Pro Tyr Ile Asp Phe Lys Arg Leu Lys Glu Lys Arg Ser Ser Leu
            195   200   205

Val Thr Lys Ile Tyr
            210

<210> 76
<211> 576
<212> DNA
<213> Mouse

<400> 76
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agggaagagg aagaggaatc cagacccagg aagctgtgcg gcaggcacct gctgatagaa   120

gttataaaac tctgtggcca aagtgactgg agccggttcg agatggagga gcaaagtcct   180

atgacacagt tctttcccca ctactcacgc aagggcaaag ccttcaaccc tcacccttct   240

tcctccgcct ggagaagatt cacaaaccca gtccctgcag gcgtctctca gaagaaagga   300

acacacactt gggagcctca gtcactgccc gactatcagt ttgaaaagac ggagttgctt   360

cctaaggcaa gagtgttttc ataccacagt ggcaagccct atgttaagag cgtacaactt   420

cagaagaaaa gcacgaacaa aatgaatacc ttcagaagtt tattttgggg gaatcattcc   480

caaaggaaac gcagaggctt tgcagataag tgctgtgtga taggatgcac caaagaagag   540

atggccgtcg cgtgcctccc ctttgttgat ttttaa   576

<210> 77
<211> 191
<212> PRT
<213> Mouse

<400> 77

Met Lys Gln Leu Cys Cys Ser Cys Leu Leu Trp Leu Gly Leu Leu Leu
1   5   10   15

Thr Pro Phe Ser Arg Glu Glu Glu Glu Ser Arg Pro Arg Lys Leu
            20   25   30

Cys Gly Arg His Leu Leu Ile Glu Val Ile Lys Leu Cys Gly Gln Ser
            35   40   45

Asp Trp Ser Arg Phe Glu Met Glu Glu Gln Ser Pro Met Thr Gln Phe
        50   55   60

Phe Pro His Tyr Ser Arg Lys Gly Lys Ala Phe Asn Pro His Pro Ser
65   70   75   80

Ser Ser Ala Trp Arg Arg Phe Thr Asn Pro Val Pro Ala Gly Val Ser
                85   90   95

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Gln Lys Lys Gly Thr His Thr Trp Glu Pro Gln Ser Leu Pro Asp Tyr
            100                 105                 110

Gln Phe Glu Lys Thr Glu Leu Leu Pro Lys Ala Arg Val Phe Ser Tyr
        115                 120                 125

His Ser Gly Lys Pro Tyr Val Lys Ser Val Gln Leu Gln Lys Lys Ser
    130                 135                 140

Thr Asn Lys Met Asn Thr Phe Arg Ser Leu Phe Trp Gly Asn His Ser
145                 150                 155                 160

Gln Arg Lys Arg Arg Gly Phe Ala Asp Lys Cys Cys Val Ile Gly Cys
                165                 170                 175

Thr Lys Glu Glu Met Ala Val Ala Cys Leu Pro Phe Val Asp Phe
            180                 185                 190
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<210> 78
<211> 567
<212> DNA
<213> Rat

<400> 78
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gttataaaac tctgtggcca aaatgactgg agccggttct cgatggaaga gcaaagtcct     180

atgacagagt tggttcccca atatacacgg aaagtcaaaa ccttcaaccc tcaccggtcc     240

tcctcctcct ggggaagatt cacaaaccca ggcgtctccc agaagaaagc aacacacact     300

tgggaatctc agtcactgcc caactatcag cttaaaaagg aggagctgct tccgaagaca     360

ggagtgcatt cataccacgg tggcaagccc tatgtgaaga gtgtaaaatt tcagaagaaa     420

aacactgaca aaatgagtac cttcagcggc ttattttggg ggaaccatcc ccagaggaag     480

cgcagaggtt cgcagataaa tgctgtgct ataggtgct ccaaagagga gctggccgtc       540

gcatgccttc cgtttgttga ttttttaa                                        567
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<210> 79
<211> 188
<212> PRT
<213> Rat

<400> 79
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Met Lys Gln Leu Cys Cys Ser Cys Leu Leu Trp Leu Gly Leu Leu Leu
1               5                   10                  15

Ala Pro Phe Ser Gln Glu Gln Glu Glu Val Thr Ser Pro Thr Lys Leu
            20                  25                  30

Cys Gly Arg Asp Leu Leu Val Glu Val Ile Lys Leu Cys Gly Gln Asn
    35                  40                  45
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Asp Trp Ser Arg Phe Ser Met Glu Glu Gln Ser Pro Met Thr Glu Leu
    50   55   60

Val Pro Gln Tyr Thr Arg Lys Val Lys Thr Phe Asn Pro His Arg Ser
65   70   75   80

Ser Ser Ser Trp Gly Arg Phe Thr Asn Pro Gly Val Ser Gln Lys Lys
            85   90   95

Ala Thr His Thr Trp Glu Ser Gln Ser Leu Pro Asn Tyr Gln Leu Lys
            100   105   110

Lys Glu Glu Leu Leu Pro Lys Thr Gly Val His Ser Tyr His Gly Gly
        115   120   125

Lys Pro Tyr Val Lys Ser Val Lys Phe Gln Lys Lys Asn Thr Asp Lys
    130   135   140

Met Ser Thr Phe Ser Gly Leu Phe Trp Gly Asn His Pro Gln Arg Lys
145   150   155   160

Arg Arg Gly Phe Ala Asp Lys Cys Cys Ala Ile Gly Cys Ser Lys Glu
            165   170   175

Glu Leu Ala Val Ala Cys Leu Pro Phe Val Asp Phe
            180   185

<210>   80
<211>   52
<212>   PRT
<213>   Human

<400>   80

Lys Trp Lys Asp Asp Val Ile Lys Leu Cys Gly Arg Glu Leu Val Arg
1   5   10   15

Ala Gln Ile Ala Ile Cys Gly Met Ser Thr Trp Ser Arg Pro Tyr Val
            20   25   30

Ala Leu Phe Glu Lys Cys Cys Leu Ile Gly Cys Thr Lys Arg Ser Leu
        35   40   45

Ala Lys Tyr Cys
        50

<210>   81
<211>   53
<212>   PRT
<213>   Human

<400>   81

Asp Ser Trp Met Glu Glu Val Ile Lys Leu Cys Gly Arg Glu Leu Val
Page 47

1   5   10   15

Arg Ala Gln Ile Ala Ile Cys Gly Met Ser Thr Trp Ser Gln Leu Tyr
            20   25   30

Ser Ala Leu Ala Asn Lys Cys Cys His Val Gly Cys Thr Lys Arg Ser
        35   40   45

Leu Ala Arg Phe Cys
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<210>   82
<211>   51
<212>   PRT
<213>   Human

<400>   82
Pro Thr Pro Glu Met Arg Glu Lys Leu Cys Gly His His Phe Val Arg
1   5   10   15

Ala Leu Val Arg Val Cys Gly Gly Pro Arg Trp Ser Thr Glu Ala Asn
            20   25   30

Pro Ala Arg Tyr Cys Cys Leu Ser Gly Cys Thr Gln Gln Asp Leu Leu
        35   40   45

Thr Leu Cys
    50

<210>   83
<211>   52
<212>   PRT
<213>   Human

<400>   83
Glu Ser Leu Ala Ala Glu Leu Arg Gly Cys Gly Pro Arg Phe Gly Lys
1   5   10   15

His Leu Leu Ser Tyr Cys Pro Met Pro Glu Lys Ser Gly Arg His Arg
            20   25   30

Phe Asp Pro Phe Cys Cys Glu Val Ile Cys Asp Asp Gly Thr Ser Val
        35   40   45

Lys Leu Cys Thr
    50

<210>   84
<211>   45
<212>   PRT
<213>   Human

<400>   84
Lys Glu Ser Val Arg Leu Cys Gly Leu Glu Tyr Ile Arg Thr Val Ile

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1                  5                    10                   15
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Tyr Ile Cys Ala Ser Ser Arg Trp Gln Asp Leu Gln Thr Leu Cys Cys
            20   25   30

Thr Asp Gly Cys Ser Met Thr Asp Leu Ser Ala Leu Cys
        35   40   45

<210>   85
<211>   57
<212>   PRT
<213>   Human

<400>   85

Ile Ser Ser Ala Arg Lys Leu Cys Gly Arg Tyr Leu Val Lys Glu Ile
1   5   10   15

Glu Lys Leu Cys Gly His Ala Asn Trp Ser Gln Phe Gly Tyr Ser Glu
            20   25   30

Lys Cys Cys Leu Thr Gly Cys Thr Lys Glu Glu Leu Ser Ile Ala Cys
            35   40   45

Leu Pro Tyr Ile Asp Phe Lys Arg Leu
        50   55

<210>   86
<211>   51
<212>   PRT
<213>   Human

<400>   86

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1   5   10   15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Gly Ile
            20   25   30

Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn
            35   40   45

Tyr Cys Asn
    50

<210>   87
<211>   70
<212>   PRT
<213>   Human

<400>   87

Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1   5   10   15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly

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                  20                    25                    30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                    40                    45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                    55                    60

Lys Pro Ala Lys Ser Ala
65                    70
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<210>   88
<211>   67
<212>   PRT
<213>   Human

<400>   88
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1               5                   10                    15

Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
            20                    25                    30

Ser Arg Val Ser Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
        35                    40                    45

Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
    50                    55                    60

Lys Ser Glu
65
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